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CALCIFICATION OF THE MYOCARDIUM

A Pathologic Study of Thirteen Cases

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CALCIFICATION of the muscle fibers of the heart is rarely observed, but it is not unusual to see deposits of calcium within large myocardial scars. Brown and Evans,[1] in a review of the literature before 1940, were able to find only 12 cases of calcification of myocardial scars, although many pathologists can recall similar instances (unpublished) from their own experience. It was the first radiologic demonstration of an anatomically proved calcified ventricular aneurysm in 1919 which led Scholz [2] to review the subject thoroughly in 1924. Essentially this review of the material at the Army Institute of Pathology merely confirms his observations on the cases collected from the literature. It was not always clear from his account of the reported cases whether or not the mineral deposit had involved muscle fibers themselves or the scar tissues which had replaced necrotic foci. This distinction has been drawn in the present series and an effort has been made to ascertain, if possible, the factors responsible for it.

Our attention was first called to calcification of the myocardium by an interesting case, briefly outlined here but presented in detail in another context.[3] While running an obstacle course, a white soldier, 23 years of age, struck his chest forcibly against a wall and was thrown to the ground. Though he completed the run, he staggered and suffered from precordial oppression. He was hospitalized promptly in a state of severe shock. There was ventricular tachycardia of 264 per minute. Therapeutic measures directed at ending both the shock and the tachycardia were not effective for twenty-six hours. Subsequently, severe and stubborn oliguria set in, which persisted until death, seven days later, caused by uremia and left ventricular failure. During the period of hospitalization, repeated electrocardiograms had shown evidence of

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1. Brown, C. E., and Evans, W. D.: Am. Heart J. **19**:106, 1940.
2. Scholz, T.: Arch. Int. Med. **34**:32, 1924.
3. Gore, I.: The Question of Traumatic Heart Disease, to be published.

severe myocardial damage; clinically a gallop rhythm had been observed from the fourth day onward.

Since the patient had appeared to be in good health, had fulfilled the rigid physical requirements for Officer Candidate School and had experienced no difficulty from participation in strenuous activity, the heart disease was regarded as traumatic.[4] However, pathologic examination promptly excluded that possibility, revealing myocarditis which clearly had preceded the traumatic incident. There were large macroscopic areas of myocardial necrosis; under the microscope many of the

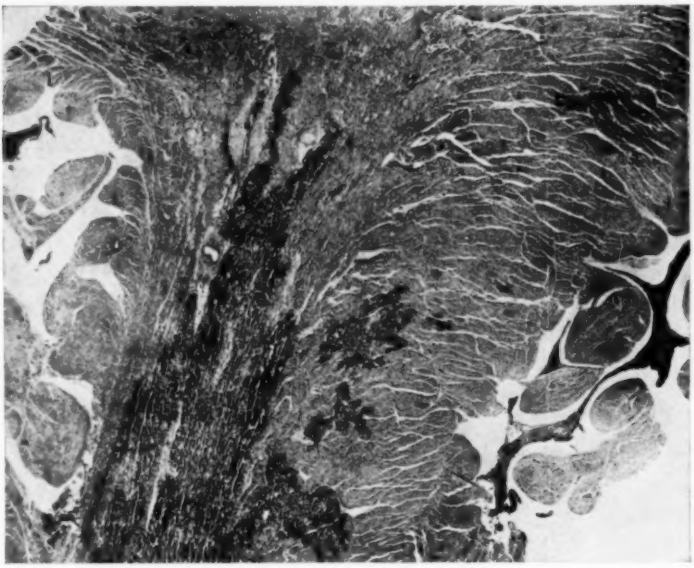

Fig. 1 (case 2, AIP Neg. 75716).—Microtessar photograph of a section through the posterior part of the interventricular septum. The darkly stained areas represent deposits of calcium in degenerated portions of the myocardium. Hematoxylin and eosin; × 10.

involved muscle fibers were heavily incrusted with calcium, verified by the von Kossa stain (fig. 1). Such areas had imparted a gritty sensation to the knife blade. In interpreting this unusual finding, it seemed reasonable to assume that the renal failure, a result of lower nephron nephrosis, had facilitated deposition of calcium on initially necrotic heart muscle. A review of material with similar deposits within the myocar-

4. Warburg, E.: Brit. Heart J. **2**:271, 1940. Arenberg, H.: Ann. Int. Med. **19**:326, 1943.

dium was undertaken at the Army Institute of Pathology in order to collect evidence by which the validity of the premise formulated in this one case could be evaluated.

MATERIAL AND METHODS

Records and tissues in which the reviewing pathologist had noted the presence of calcific deposits within the myocardium were available from 13 autopsies. In addition to the slides routinely stained with hematoxylin and eosin, selected sections were prepared by the von Kossa technic for calcium, and the Turnbull and prussian blue reactions for iron. Stains for iron were done because of the intimate relation between iron and calcific deposits which is so frequently cited, and also in order to investigate the possibility that the breakdown of myoglobin, specifically, might be a contributory factor in causing the deposition of calcium.

OBSERVATIONS

The results of this investigation together with other pertinent data are listed briefly in the accompanying table. Case 2 (AIP Acc. 88679) will be recognized as the one just described.

The one factor common to all cases was the presence of a pathologic process leading to myocardial necrosis. Calcification was never observed in the absence of necrosis of muscle, but a varying quantity of uncalcified degenerated muscle could be demonstrated in every case. The causes of the degenerative myocardial process were ischemia in 4 cases (9, 10, 11 and 12), myocarditis of infectious origin in 3 cases (1, 4 and 5) and a combination of ischemic and infectious factors in 2 cases (3 and 9). In 4 cases (2, 6, 7 and 13) the cause of the degenerative myocardial process was unknown. The duration of the terminal illness, a clinical figure, not necessarily an accurate measure of the predisposing process, varied from one day to eleven months, but in each instance the degenerative process in the heart, judged histologically, appeared to be relatively recent, measurable in days or, at most, weeks.

The reactions with the special stains demonstrated the fallacy of a practice common among pathologists, i.e., the identifying of an incrustation or deposit as calcium from the deep purple color which it assumes in hematoxylin and eosin preparations. In 6 cases both iron and calcium were demonstrated (figs. 1, 2, 3 and 4); in 4 the entire deposit proved to be calcium (fig. 5) and in 3 iron only was found (fig. 6). The two deposits were not always coextensive, and consequently a clearcut relation between them could not be established.

The kidneys were sufficiently diseased in 11 cases to cause significant functional impairment, demonstrated clinically by blood nitrogen retention in 8. The renal involvement was of diverse pathologic types: There were 4 examples of lower nephron nephrosis (cases 1, 2, 4 and 6), 4 of severe arteriolar nephrosclerosis (cases 8, 9, 10 and 12), 2 of acute and subacute glomerular nephritis (cases 7 and 13) and 1 of severe focal embolic glomerular disease (case 3). Azotemia of prerenal origin could be reasonably assumed in a twelfth case (5), that of a patient who died in congestive heart failure as a result of diphtheritic myocarditis. The sole patient in whom there was no reason to suspect renal excretory inadequacy was an infant of 4 months (case 11) with a history of excessive intake of vitamin D over a period of two and one-half months.

Foci of calcification outside the heart were observed in 5 cases. Chalky deposits were associated with acute pancreatic fat necrosis in case 8; in case 6 necrotic cells in a traumatic infarct of the liver were incrusted; focal calcification of the interalveolar septums of the lungs was present in cases 7, 11 and 12, and in

Case	AIP Account Number	Age, Yr., Race, Sex	Nature of the Myocardial Lesion	Minerals Demonstrated Histologically: Calcium	Minerals Demonstrated Histologically: Iron	Factors of Etiologic Significance in the Production of the Myocardial Lesion	Azotemia	Cause of Azotemia	Blood Chemistry, Mg. per 100 Cc.	Associated Pathologic Changes	Duration of Terminal Illness
1	86,202	38 N M	Mineralization involves muscle fibers in widespread foci of acute degeneration, there is a mild reactive mononuclear cell infiltration	+	+	Lobar pneumonia	+	Lower nephron nephrosis (sulfa effect)	Nonprotein nitrogen, 400 Creatinine, 7.3	Inactive syphilitic aortitis, focal hepatic necroses, terminal pneumococcic sepsis	9 days
2	88,679	23 W M	Acute myocarditis; widespread muscle degeneration with a prominent infiltrate of granulocytes and mononuclear cells; extensive mineralization of necrotic fibers	+	±	Unknown. Histologically, the cardiac lesion clearly antedates the acute symptoms which occasioned hospitalization	+	Lower nephron nephrosis	Nonprotein nitrogen, 186 Phosphorus, 8.8 Calcium, 9.7	Central zonal necrosis of the liver	? Death occurred on the 9th hospital day
3	91,096	21 N F	Widespread foci of ischemic necrosis, old and recent; mineral incrustation has involved many of the most recently damaged fibers	+	–	Subacute bacterial endocarditis involving the mitral valve	+	Multiple renal infarcts, focal embolic glomerulonephritis	None	Infarcts of lung and spleen	6 mo.
4	92,264	43 W M	Small foci of acute myocardial necrosis with moderate granulocytic infiltration; mineralization has involved many of the degenerate fibers	+	–	Bronchopneumonia, acute hepatitis	+	Lower nephron nephrosis	Nonprotein nitrogen, 110-210 Creatinine, 16 CO_2, 61-71 vols. %	Acute hepatitis with central zonal necrosis, bronchopneumonia	15 days
5	109,334	26 W M	Widespread foci of muscle necrosis with prominent leukocytic reaction; some of the involved fibers show mineral incrustation	–	+	Pharyngeal diphtheria	+	Cardiac failure, prerenal azotemia	None	Congestive heart failure	12 days
6	118,951	21-39 W M	Focal necroses and fibrosis with surrounding mixed leukocytic response; mineralization of many of the acutely necrotic fibers	–	+	Unknown. Histologically the cardiac changes easily antedate the terminal clinical episode	+	Lower nephron nephrosis	None	Calcification of necrotic cells in liver infarcts; multiple shell fragment wounds; surgical amputation of right lower leg	4 days
7	127,962	27 W M	Tiny scattered foci of acute myocardial degeneration with scant mononuclear cell reaction; many of the necrotic fibers are heavily mineralized	+	+	Unknown. The cardiac process is much more recent and acute than the renal disease	+	Subacute glomerulonephritis	Nonprotein nitrogen, 132 Calcium, 7.6 CO_2, 56 vols. %	Bronchopneumonia; focal mineralization of pulmonary interalveolar septums	5 mo.
8	144,674	52 W M	Numerous tiny foci of acute necrosis; most of the fibers are heavily incrusted; stromal reaction is sparse and predominately mononuclear; necrotic fibers at the margins of occasional pyemic micro abscesses also show mineral deposition	+	±	Bronchopneumonia and terminal pyemia; coronary sclerosis and circulatory failure	+	Severe arteriolar nephrosclerosis	Nonprotein nitrogen, 137-258 CO_2, 78-84 vols. %	Bronchopneumonia, acute pancreatitis with chalky deposits in fat necroses, terminal sepsis with pyemic abcesses of kidney	7 mo.
9	183,518	47 W M	Healing myocardial infarct with terminal extension; calcification has involved the acutely necrotic muscle though the process is sufficiently recent to be without leukocytic response	+	–	Severe coronary sclerosis and thrombosis of anterior branch of left C.A., cardiac decompensation	+	Severe arteriolar nephrosclerosis; circulatory failure	Nonprotein nitrogen, 146 Creatinine, 5.8	Bronchopneumonia, congestive heart failure	Mild decompensation, 3 yr.; severe decompensation, 6 wk.
10	184,634	61 W M	Old and recent myocardial infarcts; acutely necrotic fibers at periphery of infarcts are heavily incrusted; intense granulocytic response	+	±	Severe coronary sclerosis, pneumonia and circulatory failure	+	Severe arteriolar nephrosclerosis; circulatory failure	Blood urea nitrogen, 100 CO_2, 45 vols. %	Bronchopneumonia, congestive heart failure	21 days
11	187,787	4/12 W M	Foci of ischemic necrosis with mineralization of many of the fibers; inflammatory reaction absent	+	–	Coronary thrombosis left C.A. secondary to medial calcification of coronary arteries; hypervitaminosis D (3,400 units daily—3 mo.)	–	None	None	Acute congestive heart failure, calcification involving the elastica of the systemic arteries and foci, interalveolar septums of lungs	1 day
12	188,406	67 W M	Focal acute muscle degeneration with prominent mixed type of leukocytic response; most of the necrotic fibers are heavily mineralized; fairly prominent myolysis indicates duration of at least 1 week	–	+	Moderately severe coronary sclerosis, bronchopneumonia	+	Severe arteriolar nephrosclerosis; extensive alkalitic nephrosis	None	Chronic duodenal ulcer, pyloric obstruction, gastric retention, terminal bronchopneumonia, focal calcification of interalveolar septums of lungs	11 mo.
13	196,797	37	Conspicuous and numerous but small foci of mineralization superimposed upon necrotic muscle; there is only slight associated inflammatory infiltration	+	+	Unknown. Histologically the cardiac changes are too recent to correlate with any of the other findings	+	Acute glomerulonephritis	Nonprotein nitrogen, 102-173	Hemochromatosis, portal cirrhosis with liver failure	Cirrhosis, 3 yr. Nephritis, 1-2 mo.

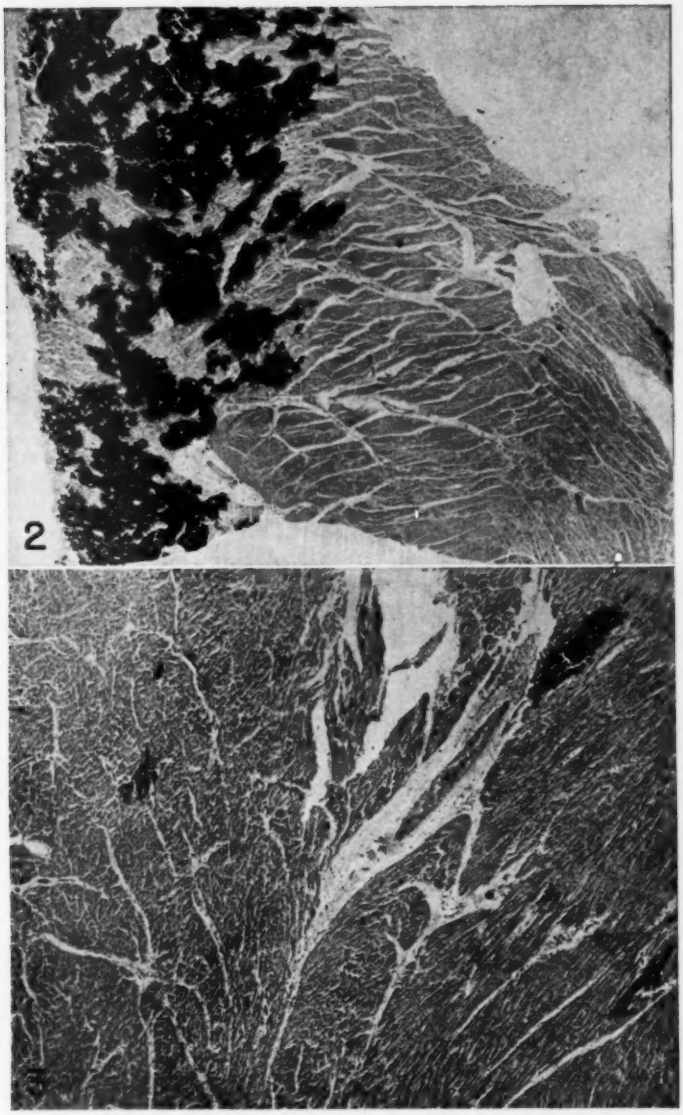

Fig. 2 (case 1, AIP Neg. 86202).—A portion of the left ventricular wall stained with hematoxylin and eosin. The darkly stained coalescent areas in the outer myocardium represent calcified necrotic muscle fibers. × 12.

Fig. 3 (case 13, AIP Neg. 196797).—Observe the three darkly stained areas representing deposits of calcium in necrotic foci of heart muscle. Hematoxylin and eosin; × 28.

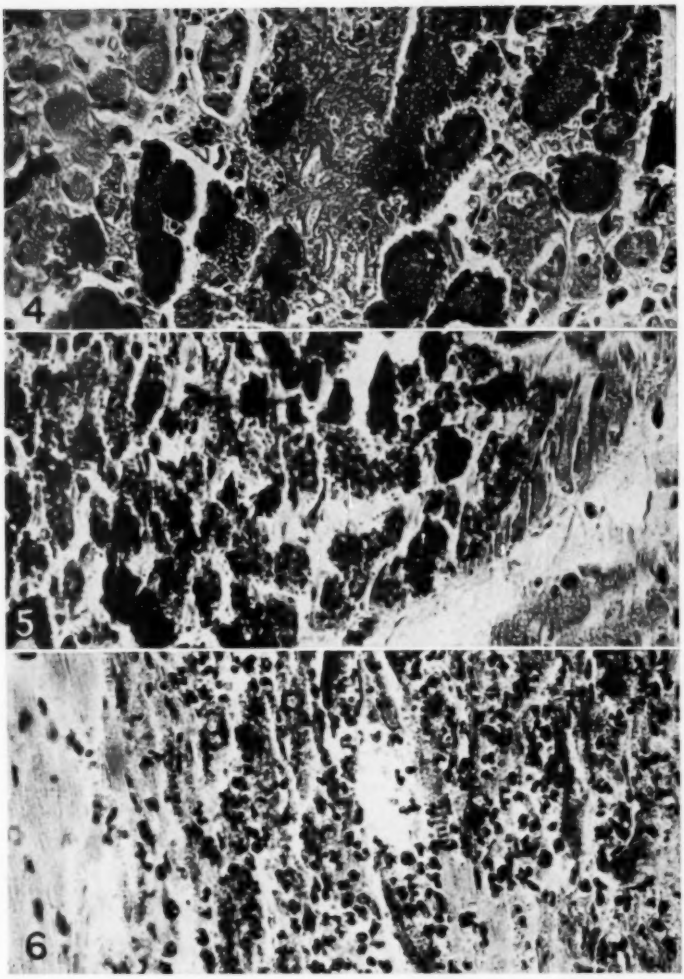

Fig. 4 (case 2, AIP Neg. 86202).—Deposits of calcium on necrotic myofibrils account for their dark stain and granular character. To the left of the center in the upper portion of the photograph, and to the right in the lower portion, uncalcified necrotic fibers are to be noted. Hematoxylin and eosin; × 355.

Fig. 5 (case 3, AIP Neg. 91096).—Observe that the granular deposits of darkly stained calcium do not involve the necrotic myofibrils uniformly. Hematoxylin and eosin; × 355.

Fig. 6 (case 5, AIP Neg. 109334).—The deposits of darkly stained granular material on the necrotic myofibrils represent iron. The leukocytic reaction provides a clue as to the duration of the degenerative process. Hematoxylin and eosin; × 300.

case 11 (that of the infant previously referred to) there was also widespread calcification of the internal elastic lamina of the systemic arteries.

Unfortunately, there were only 2 patients in whom blood calcium levels had been determined before death. In case 2 the value was within normal limits (9.7 mg. per hundred cubic centimeters), but the corresponding phosphorus value was significantly elevated to 8.8 mg. A figure of 7.6 mg. of calcium in case 7 is distinctly low but cannot be properly evaluated without knowledge of the plasma protein level, which is likely to be reduced in subacute glomerular nephritis.

COMMENT

Pathologic calcification is generally considered to be either dystrophic or metastatic in nature,[5] the former being by far the more frequent. As Wells [6] has stated, "any area of dead tissue that is not infected and that is so large or so situated that it cannot be absorbed, will probably become infiltrated with lime salts." Calcification is common, particularly in old atheromatous lesions of the aorta; it characterizes the medial sclerosis of peripheral arteries. Deposits of calcium in foci of necrosis occur sufficiently often to be considered of some significance, at least in the roentgenologic diagnosis of such conditions as tuberculosis, histoplasmosis and toxoplasmosis. Hemorrhagic extravasates seem to be particularly susceptible to calcification, and examples often seen clinically are myositis ossificans and the deposits which occur in degenerating and hemorrhagic "adenomas" of the thyroid gland. Precipitation of lime salts in hyaline scars, though necrosis is not involved, is also regarded as a form of dystrophic calcification. It has been related to the low production of carbon dioxide in a slowly metabolizing tissue, which permits development of a local zone of relative alkalinity and, consequently, local reduction of calcium solubility. Dystrophic calcification may proceed without variation of the physiologic levels of any of the chemical constituents of the blood.

On the contrary, metastatic calcification is associated with increased availability of calcium. As Mulligan [7] has emphasized, this type of mineralization has been found with bone destructive lesions, with hyperparathyroidism and hypervitaminosis D and with renal insufficiency. Elevated levels of blood phosphorus occur in renal insufficiency; calcium may remain unchanged, as in case 2, or may even be depressed, as in case 7. However, secondary parathyroid hyperplasia and hyperactivity associated with chronic renal disease may, at times, result in hypercalcemia. Metastatic calcification, unlike the dystrophic type, has a characteristic distribution.[8] The chemical structure of elastic tissue serves

5. Karsner, H. T.: Human Pathology, ed. 6, Philadelphia, J. B. Lippincott Company, 1942.

6. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

7. Mulligan, R. M.: Arch. Path. **43**:177, 1947.

to explain involvement of arterial and endocardial (right auricle) elastica by metastatic lime salt deposits.[8] In the lungs the veins rather than the arteries are involved,[9] indicating the influence that high oxygen and low carbon dioxide tensions exert through their effect on the hydrogen ion concentration. Wells provided the explanation, since generally accepted, for the localizing of metastatic mineral deposits in and about such tissues as pulmonary alveolar septums, gastric mucosa and renal tubular epithelium. The physiologic activity of these structures results in local alkalinity of tissue by virtue of the excretion or secretion of acid.[10] Confirming the importance of an alkaline reaction in permitting or facilitating lime deposits, Mulligan and Stricker [11] found that lesions were more severe in dogs receiving alkaline salts to complement the effect of excessive doses of vitamin D which were used to produce metastatic calcification. Though heavily mineralized tissues are obviously incapable of the complex chemical and physical interchanges essential to life, it seems likely that the initial deposition occurs on vital tissues and constitutes still another distinction from dystrophic calcification.[12] The selective localization of metastatic deposits of lime salts is, therefore, a function of both normal structure and physiologic activity in the presence of systemically increased availability of calcium; dystrophic calcification owes its occurrence purely to local pathologic changes and has no characteristic distribution.

A brief discussion of the chemical basis of pathologic calcification seems warranted. Normally the blood level of calcium represents chemical saturation.[13] The relation of calcium to phosphate is of prime importance to the occurrence of either ossification or calcification since, as has been amply verified, the chemical bulk of such deposits consists of the insoluble form of tricalcium phosphate.[14] In such a saturated solution of a poorly soluble substance, the relations of the concentrations of ionic calcium and phosphate, molecular calcium phosphate and the undissolved salt, following the laws of ionic equilibrium, explain the reciprocal alterations usually observed in the blood levels of these two substances.[15] An excess of calcium or of phosphate or of both, such as occurs in conditions producing metastatic calcification, shifts the equilibrium toward the formation of more insoluble salt. Decreasing hydrogen ion concentration causes a similar shift in the equilibrium, since there is decreased solubility of molecular calcium phosphate. Dystrophic calcification, of course,

8. Hass, G. M.: Arch. Path. **27**:334 and 583, 1939.
9. Wells.[6] Karsner.[5] Mulligan.[7]
10. Wells.[6] Mulligan.[7]
11. Mulligan, R. M., and Stricker, F. L.: Am. J. Path. **24**:451, 1948.
12. (*a*) Barr, D. P.: Physiol. Rev. **12**:592, 1932. (*b*) Scholz.[2] (*c*) Wells.[6]
13. (*a*) Logan, M. A.: Physiol, Rev. **20**:522, 1940. (*b*) Barr.[12a]
14. Wells.[6] Logan.[13a]
15. Howland, J., and Kramer, B.: Tr. Am. Pediat. Soc. **34**:204, 1922. Logan.[13a]

obeys the same natural laws, but in a manner less easily explained. Superficially it would seem that the elevation of hydrogen ion concentration which accompanies necrosis would prevent the deposition of lime salts, and perhaps this is one of the reasons dystrophic calcification is not more common than it is. However, the enzymic degradation of tissues, with their content of nucleotides, phospholipids and phosphoric esters, results in a local excess of phosphate which could easily cause calcium to be precipitated from its physiologic levels. Rapid absorption of such products understandably precludes that possibility. The activity of phosphatase, which is regarded as significant in ossification, does not appear to be essential to the explanation of pathologic calcification. In discussing this problem, Wells [6] had questioned that the phosphoric acid contents of tissues have any relation to the frequency with which they exhibit calcific deposits. He had found experimentally that boiled tissues rich in nucleoprotein and phosphoric acid implanted into the abdominal cavity of rabbits showed no more extensive calcification than did tissues poor in these substances. This observation may be interpreted equally well as evidence that the potential phosphate content of any tissue is adequate to produce deposition of calcium by this mechanism and that the additional quantities present in tissues rich in nucleoprotein are superfluous, provided that the blood calcium remains at a physiologic level. It is obvious that any condition favoring the development of metastatic calcification would, by increasing the availability of calcium, simultaneously favor and accelerate the dystrophic variety.

Inasmuch as necrosis was invariably present and seemed to be the *sine qua non* of myocardial calcification in these cases, the process must be considered dystrophic. However, in case 11, an instance of vitamin D intoxication, autopsy showed, in addition, typical metastatic calcification of the systemic arteries and lungs, and in the remaining cases, without exception, there was evidence of renal excretory inadequacy and azotemia. It is interesting that extensive renal disease was also a factor in many of the cases collected by Scholz.[2] He noted that the lesions of the kidneys were correlated with a degenerative process of varied causation in the heart, but his explanation, limited by the primitive knowledge of calcium and phosphorus metabolism, would be regarded as inadequate today. As Mulligan [7] has so capably outlined, the changes of calcium and phosphate balance produced by renal failure favor the metastatic deposition of lime salts. As a matter of fact, such foci were present in the lungs in 2 of our cases (7 and 12). Failure to demonstrate them in the other cases of this series is not surprising, for, to judge from the examples observed, the formation of these lesions is usually gradual. The point remains that in these cases necrotic tissues were exposed to conditions which favored and accelerated the deposition of

lime salts. Necrotic heart muscle and its products are ordinarily absorbed too rapidly to permit calcification. In diphtheritic myocarditis, Gore [16] has shown that myolysis, which begins early in the second week, is fairly complete by the end of the third week. The resorptive process may be somewhat delayed in the central part of large infarcts, relatively remote from an effective blood supply, but access of calcium salts is limited for the same reason; this is illustrated in case 10, in which a heavier incrustation occurs at the periphery of the infarct. In infants, however, in whom there is a physiologic elevation of blood phosphorus, and in small experimental animals with active metabolism it is not uncommon to see calcific deposits in areas of myocardial necrosis. Kaunitz,[17] reporting on an anomalous origin of the left coronary artery, gave an example of myocardial calcification occurring in an infant and listed several other cases in which the fundamental lesion was ischemic. The virus-induced myocardial changes produced by Pearce [18] in rabbits were calcified within a few days after the introduction of the causative infectious agent.

Perhaps because clinical evidence of calcification is slow to appear in such diseases as pulmonary tuberculosis, the rapidity with which it sometimes takes place is not generally appreciated. In the experimental work of Pearce [18] it occurred within a few days, though no condition was described which might have caused an acceleration. In case 6 of this series, liver cells which could not have been necrotic longer than four days were already heavily calcified. In the other cases the time relations were not so clearly defined, but the failure to demonstrate significant myolysis in the involved degenerated areas of the heart indicated the recency of the necrosis, which of necessity had preceded the deposition of mineral salt. In case 8, for example, many of the necrotic and secondarily calcified foci were related to pyemia which developed terminally. Wells [6] observed deposition of mineral salts as soon as four hours after the occurrence of pancreatic fat necrosis; but in that condition the precipitate was formed of insoluble calcium soaps rather than phosphates.

The nature of the processes leading to myocardial necrosis needs little comment. The relationship of infectious diseases and of certain drugs (sulfonamides, in particular) to myocarditis has been adequately covered in the literature.[19] Ischemia as a cause of cardiac degeneration requires no documentation. Yet, even when all the factors have been considered, there still remain 4 of our cases in which the pathogenesis

16. Gore, I.: Am. J. M. Sc. **215**:257, 1948.
17. Kaunitz, P. E.: Am. Heart J. **33**:182, 1947.
18. Pearce, J. M.: Arch. Path. **34**:319, 1942.
19. Gore, I.: Am. Pract. **1**:292, 1947. Gore, I., and Saphir, O.: Am. Heart J. **34**:827, 1947. Saphir, O.: Arch. Path. **32**:1000, 1941; **33**:88, 1942.

of the cardiac lesions was obscure. In 2 cases (7 and 13) of subacute and glomerular nephritis, respectively, the type of myocardial change was identical: multiple, scattered tiny foci of acute necrosis which obviously must have occurred late in the course of the rather prolonged terminal illness (fig. 3). The lesions are to be distinguished from the serous type of myocarditis which may complicate the course of acute or subacute glomerular nephritis.[20] They appear to be similar to the miliary necroses and foci of interstitial inflammation which Solomon, Roberts and Lisa [21] described in 25 of the hearts of 26 uremic patients who died of circulatory or congestive heart failure. Experimentally, Smadel and Farr [22] have observed the lesions of acute myocardial necrosis in 14 of 20 rats with nephrotoxic nephritis and protracted renal insufficiency. Certain rather characteristic electrocardiographic disturbances in uremia have been related clinically to potassium intoxication, but in the cases studied clinically and at autopsy by Langendorf and Pirani [23] focal myocardial necroses were not observed. There is the possibility that in uremia the accumulation of nonvolatile metabolites eventually reaches a point at which it becomes incompatible with the survival of a metabolically highly active and unresting tissue. Although the available information is inadequate as yet to restate this premise in specific chemical terms, it is a thesis for which the well proved laws of mass action and chemical ionic equilibrium demand support. The focal rather than general distribution of lesions produced in this fashion may be accounted for by minor differences of the cells within the tissue and by slight variations in their relations to the circulation. If we may be permitted to draw an analogy, Himsworth [24] has summarized the data which relate certain focal lesions of the liver to variations in the distribution of the blood. Systemic conditions, such as hyperpyrexia,[25] thiamine deficiency [26] and lack of potassium [27] have been shown to produce lesions of focal distribution in the heart.

We were unable to draw any conclusions regarding the relationship of calcium and iron deposition. Hektoen [28] was among the first to call attention to the tendency of calcium-iron incrustation to involve elastic

20. Gore, I., and Saphir, O.: Am. Heart J. **36**:390, 1948.

21. Solomon, C.; Roberts, J. E., and Lisa, V. R.: Am. J. Path. **18**:729, 1942.

22. Smadel, J. E., and Farr, L. E.: Am. J. Path. **15**:199, 1939.

23. Langendorf, R., and Pirani, C. L.: Am. Heart J. **33**:282, 1947.

24. Himsworth, H. P.: The Liver and Its Diseases, Cambridge, Mass., Harvard University Press, 1947.

25. Gore, I., and Isaacson, N.: The Pathology of Hyperpyrexia, Am. J. Path., to be published.

26. Rinehart, J. F.; Greenberg, L. D., and Friedman, M.: Am. J. Path. **23**:879, 1947.

27. Follis, R. H., Jr.: The Pathology of Nutritional Disease, Springfield, Ill., Charles C Thomas, Publisher, 1948.

28. Hektoen, L.: J. M. Research **7**:159, 1902.

fibers in the vicinity of hemorrhages. Klotz [29] showed that the sidero-fibrotic nodules which occur in the spleen in splenic anemia often become calcified. The frequency with which either deposit occurred alone in the present material and their failure to be completely coextensive when both were present carry no conviction of an intimate association. Possibly the short duration of the process accounts for this variation from changes usually described in much older lesions. Certainly, those instances in which iron was not demonstrable offer proof that the breakdown of myoglobin in the areas of cardiac necrosis was not essential to the subsequent deposition of calcium. Cameron [30] has presented excellent reasons for doubting the specificity of the von Kossa reaction for calcium but has also noted the inconstancy of the association of iron and calcium in normal as well as in pathologic mineral deposits.

SUMMARY

In 13 cases of calcification of the myocardium at the Army Institute of Pathology, calcium deposits had been laid down on necrotic muscle fibers which ordinarily are absorbed too rapidly to permit this eventuality. However, under circumstances which favor metastatic calcification, there is an augmented and accelerated tendency toward the development of dystrophic calcification. The process in the heart muscle in these cases is therefore regarded as an example of accelerated dystrophic mineralization. In 1 case there was a history of excessive intake of vitamin D, and at autopsy there were, in addition to the cardiac lesions, widespread metastatic deposits of calcium. Azotemia occurred in all 12 of the remaining cases, being of renal origin in 11 and of prerenal origin in 1. The pathologic types of renal involvement included both glomerular and vascular renal disease, as well as lower nephron nephrosis. The laws of mass action and of ionic equilibrium of saturated solutions of poorly soluble salts explain, in the case of tricalcium phosphate, the ease with which precipitation may be induced by increases of either calcium or phosphate.

The pathogenesis of the basic myocardial lesion also varied, and in the cases studied could be related to ischemia, to an infectious process, to combinations of the two and to unknown influences. The possible influence of uremia in causing multiple tiny foci of myocardial necrosis in 2 cases was considered.

Special staining reactions for iron and calcium showed the fallacy of making a diagnosis of calcification from a preparation stained with hematoxylin and eosin. In 3 instances the deposit proved to be iron only. Calcium was present in all the others; in 6 it was associated, but not always coextensive, with iron.

29. Klotz, O.: Bull. Johns Hopkins Hosp. **27**:363, 1916.
30. Cameron, G. R.: J. Path. & Bact. **33**:929, 1930.

LIPID FRACTIONS OF HUMAN ADRENAL GLANDS

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ADRENAL lipids, subject to repeated study since Virchow [1] first noted their rich concentration, have recently acquired new interest from the evidence that cholesterol is a precursor of the corticosteroid hormones and that fluctuations in adrenal cholesterol may reflect secretory activity.[2] Early reports of variations in adrenal lipid detected by morphologic means described reduction of the anisotropic lipid of human adrenal glands in acute and chronic infection [3] and comparatively high levels of adrenal lipid in advanced arteriosclerosis, chronic nephritis and cerebral hemorrhage.[4] Similar conclusions resulted from early chemical studies, in which relatively high values of adrenal cholesterol were found in hypertension [5] and varied types of cardiovascular renal disease,[6] while septicemia and other severe infections were associated with the lowest comparative values for adrenal cholesterol.

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1. Virchow, R.: Virchows Arch. f. path. Anat. **12**:484, 1857.

2. (*a*) Long, C. N. H., in Pincus, G.: Recent Progress in Hormone Research, New York, Academic Press, Inc., 1946, vol. 1, p. 99. (*b*) Sayers, G.; Sayers, M.; White, A., and Long, C. N. H.: Proc. Soc. Exper. Biol. & Med. **52**:200, 1943; (*c*) Yale J. Biol. & Med. **16**:361, 1944.

3. (*a*) Borberg, N. C.: Skandinav. Arch. f. Physiol. **32**:287, 1915. (*b*) Herrmann, O.: Ueber Vorkommen und Veränderungen von Myelinsubstanzen in der Nebenniere, Arb. a. d. Geb. d. path. Anat. **5**:419, 1906. (*c*) Kawamura, O.: Die Cholesterinverfettung, Jena, G. Fischer, 1911; cited by Weltmann.[3d] (*d*) Weltmann, O.: Beitr. z. path. Anat. u. z. allg. Path. **56**:278, 1913.

4. (*a*) Knack, A. V.: Virchows Arch. f. path. Anat. **220**:36, 1915. (*b*) Borberg.[3a] (*c*) Weltmann.[3d]

5. Chauffard, A.; Laroche, G., and Grigaut, A.: (*a*) Compt. rend. Soc. de biol. **73**:23, 1912; (*b*) **76**:529, 1914.

6. (*a*) Fex, J.: Biochem. Ztschr. **104**:82, 1920. (*b*) Landau, M., and McNee, J. W.: Beitr. z. path. Anat. u. z. allg. Path. **58**:667, 1914. (*c*) Wacker, L., and Hueck, W.: Arch. f. exper. Path. u. Pharmakol. **71**:373, 1913. (*d*) Chauffard and others.[5]

In several instances the data reveal that analyses of adrenal glands of normal persons yielded lipid values in high ranges, comparable to those in persons suffering from hypertension and nephrosclerosis.[7]

More recent chemical studies of human adrenal lipids have indicated an inverse relationship between phospholipid and histologically demonstrable fat [8] as well as between water and lipid content.[8b] Another report claimed no simple relationship between phosphatides and total cholesterol.[9] The relatively high values of adrenal lipid and cholesterol observed in circulatory diseases, including hypertension, and the low values in infection have been confirmed [10] as in earlier studies. Hyperthermia per se has been asserted to correlate with reduction of adrenal cholesterol [11] but was actually not separable as a factor from the infection present in most instances.

In the few recent series of cases in which lipid analyses were performed on normal human adrenal glands (death was caused by violence) the small number of values reported for total lipid [8b] and cholesterol [12] have been among the highest in each series, again comparable with those found in cases of hypertension and of cardiovascular and renal disease.

In a single report [10b] nonsterol fat was said to have been found in higher total quantity in the adrenal glands of hypertensive patients than in those of normal persons, although the total amounts of ester and free cholesterol in the two groups were comparable. Lipid concentration, determined both chemically and histologically, was found to correlate well with the presence of hypertension in a recent study [13] in which the control series consisted not of normal persons but of a group of nonhypertensive patients dead of miscellaneous disorders.

In most reports [14] hypertension and other types of circulatory disease not necessarily accompanied by hypertension were similarly associated with high values of adrenal lipid and cholesterol. In a recent morphologic study of adrenal cortical lipids,[15] however, dis-

7. Weltmann.[3d] Fex.[6a] Landau and McNee.[6b]

8. (*a*) Kutschera-Aichbergen, H.: Verhandl. d. deutsch. path. Gesellsch. **20**:133, 1925. (*b*) Materna, A., and Januschke, E.: Virchows Arch. f. path. Anat. **263**:537, 1927.

9. Woodhouse, D. L.: Biochem. J. **22**:1087, 1928.

10. (*a*) Koch, K., and Westphal, K.: Deutsches Arch. f. klin. Med. **181**:413, 1937. (*b*) Liebegott, G.: Beitr. z. path. Anat. u. z. allg. Path. **109**:93, 1944. (*c*) Kutschera-Aichbergen.[8a] (*d*) Materna and Januschke.[8b] (*e*) Woodhouse.[9]

11. Ewert, B.: Upsala läkaref. förhandl. **40**:423, 1934.

12. Koch and Westphal.[10a] Liebegott.[10b] Ewert.[11]

13. Fisher, J. A., and Hewer, T. F.: J. Path. & Bact. **70**:605, 1947.

14. Fex.[6a] Landau and McNee.[6b] Wacker and Hueck.[6c] Materna and Januschke.[8b] Koch and Westphal.[10a] Ewert.[11]

15. Sarason, E. L.: Arch. Int. Med. **71**:702, 1934.

tinctly greater quantities of stainable fat were reported observed in the adrenal glands of persons who died of hypertension than in those of patients with marked arteriosclerosis without hypertension or in those of a small group of normal adults.

Although a survey of previously determined values of the major lipid components of human adrenal glands is of interest, the variety of technics, the conditions of collection and the methods of expressing

TABLE 1.—*Summary of Previously Published Values of Total Lipid and Cholesterol Concentrations of Human Adrenal Glands*

Author	Cases	Type of Cases	Mean Values and Ranges		Expressed as
			Total Lipid	Cholesterol	
Fisher and Hewer [10]	127	Hypertension and miscellaneous nonhypertensive diseases	7.2	...	Per cent wet weight of adrenal tissue
Liebegott [10b]	7	Normal	16.1	4.9	Per cent wet weight of a single adrenal gland
	10	Hypertension, cardiac compensation	12.5	3.1	
	9	Hypertension, cardiac decompensation	10.6	3.6	
	6	Nonhypertensive condition, cardiac decompensation	6.1	3.1	
	41	Acute and chronic infections	5.7	1.1	
Koch and Westphal [10a]	9	Normal	...	10.5 Range: 6.2-20.4	Per cent dry weight
	10	Hypertension	...	13.6	
	25	Infections	...	6.0	
Ewert [11]	5	Normal	...	4.99	Per cent wet weight
	12	Afebrile conditions (including cardiovascular)	...	2.89	
	17	Febrile conditions (chiefly infections)	...	0.82	
Woodhouse [9]	38	Psychiatric disorders with miscellaneous somatic diseases	47.2	...	Per cent dry weight
Materna and Januschke [8b]	12	Normal and cardiovascular disease	16.8	...	Per cent wet weight
	20	Miscellaneous	5.4	...	
	8	Infections	2.8	...	
Chauffard, A.; Laroche, G., Grigaut, A. Ann. de méd. 8: 149, 1920	3	Normal	...	5.6 5.4 4.2	Per cent wet weight
Fex [8a]	16	Miscellaneous	...	9.5 Range: 0.5 to 20.2	Per cent dry weight
Chauffard, Laroche and Grigaut [8b]	22	Miscellaneous	...	2.8 Range: 0.4 to 8.2	Per cent wet weight

data do not permit pooling of recorded analyses for purposes of generalization. Table 1 summarizes the mean and extreme values of total lipid and/or cholesterol recorded in the reports of comprehensive quantitative investigations which we have encountered in the literature.

Analyses of the adrenal glands of animals for lipid have generally revealed that lipid, particularly cholesterol, is depleted in infections and severe intoxications, as well as after trauma, hemorrhage, anoxia and violent exercise.[2e] Experimental conditions leading to excessive accumulation of adrenal lipid have been studied with much less com-

pleteness and can be briefly summarized. Diets rich in cholesterol have been repeatedly shown to produce accumulation of lipid.[6b] Splenectomy is reported to result in increased concentrations of adrenal lipid; the mean difference between the control and the splenectomized group, however, is not striking.[16] Excessive storage of cholesterol has been observed in the adrenal glands of rats following prolonged administration of a purified preparation of the adrenocorticotropic factor of the pituitary gland of the hog,[2b] although, as is now well known, the uniform effect of acute administration is to produce prominent depletion of both adrenal cholesterol and ascorbic acid, a phenomenon thought to be associated with the elaboration and secretion of adrenal corticosteroids.[2a] Finally, in rats augmentation of adrenal lipid has been found to follow bilateral nephrectomy,[17] an observation which invites comparison with the increase of serum and liver lipids observed in dogs after bilateral renal ablation or ureteral ligation.[18]

Studies of adrenal lipids in experimental hypertension and experimentally produced renal disease remain to be made.

In evaluating the significance of the quantitative alterations which occur in human adrenal lipids in the course of various diseases and disorders, it must be recalled that only a few scattered data are available which describe the normal values. For this reason, comparisons made between the relatively high values observed in hypertension, arteriosclerosis and renal disease, on the one hand, and the low values in acute infections and intoxications, on the other hand, lose much of their validity, since the values cannot be compared with adequate normal standards. Thus, the claim that adrenal lipids are actually higher than normal in the first group of diseases has in most instances been based only on comparisons made with a miscellaneous collection of autopsy material.[19] It might be suspected that such "control" adrenal glands are themselves abnormally low in lipid and that different conclusions might be drawn from the use of more adequately selected normal glands. Indeed, such information as does exist concerning the normal values for human adrenal lipid and cholesterol suggests strongly that while infectious diseases may be associated with striking depletion of adrenal lipid, hypertension and allied disorders do not actually result in elevation of adrenal lipids.[20]

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16. Parhon, C. I.; Blinov, A., and Cahane, M.: Compt. rend. Soc. de biol. **109**:239, 1932.

17. MacKay, E. M., and MacKay, L. L.: J. Exper. Med. **46**:429, 1927.

18. Winkler, A. W.; Durlacher, S. H.; Hoff, H. E., and Man, E. B.: J. Exper. Med. **77**:473, 1943.

19. Borberg.[3a] Weltmann.[3d] Knack.[4a] Chauffard.[5] Fex.[6a] Landau and McNee.[6b] Wacker and Hueck.[6c] Kutschera-Aichbergen.[8a] Fisher and Hewer.[12]

20. Weltmann.[3d] Fex.[6a] Landau and McNee.[6b] Liebegott.[10b]

The present study deals with a series of 77 pairs of human adrenal glands which were obtained at autopsies and analyzed for the major lipid fractions.

MATERIAL AND METHODS

One or both adrenal glands were removed soon after the beginning of the autopsy—usually within two hours. They were kept in a freezing compartment at —18 C. until analyzed—generally within two weeks. After being thawed at room temperature, the glands, dissected free of fat and adherent connective tissue, were weighed on a torsion balance to the nearest 0.01 Gm. Both glands were used for the analysis in the majority of cases, portions of each being taken for the determination of both water content and lipid fractions. No attempt was made to separate medulla and cortex; so all analyses refer to whole glands.

Dry weight was determined by weighing an aliquot of 2 to 5 Gm. of random fragments from both glands before and after constant weight had been attained in an oven at 70 C. The expression of lipid fractions as percentages of the dry weight of the adrenal glands avoided any influence which variable postmortem dehydration might have on lipid concentration.

The sample for analysis, usually 1 to 3 Gm., after being weighed, was ground to a pulp in a mortar and transferred quantitatively to a flask containing at least 100 cc. of a 3:1 mixture of redistilled 95 per cent alcohol and redistilled peroxide-free ether, in which it was refluxed at 70 C. for one hour. This extract was then filtered through fat-free paper into a 250 cc. volumetric flask, and the sediment of extracted adrenal pulp was washed seven times with 10 to 20 cc. portions of the alcohol-ether mixture. The filtrate was made to volume, and aliquots were taken for determination of the various lipid fractions.

Total lipid was determined by weighing the dried residue of a 50 cc. aliquot of the original extract evaporated in a water bath at 80 C.

Lipid phosphorus was determined by the method of Fiske and Subbarow [21] in a 2 cc. aliquot of the original extract, essentially as described by Man and Peters [22] for blood serum phospholipid.

Fatty acids were determined in a 25 cc. aliquot as milliequivalents of titratable acid, after saponification with potassium hydroxide, precipitation by standing, filtration through a Gooch crucible, solution of the residue in hot ethanol and titration with two hundredth normal sodium hydroxide, a method similar to that of Stoddard and Drury [23] as modified by Man and Gildea.[24]

Cholesterol, both free and total, was determined, after its separation by digitonin precipitation, by measurement of the color developed with sulfuric acid. Details of the method were similar to those described by Bogdanovitch and Man [25] except that colorimetric measurement [26] was utilized instead of gravimetric determination. Free cholesterol was determined in an aliquot of the original lipid extract; total cholesterol was determined in the aliquot used for the measurement of fatty acids, after saponification, separation and solution in ethanol.

All analyses were made on duplicate aliquots taken from the original alcohol-ether extract.

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21. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.
22. Man, E. B., and Peters, J. P.: J. Biol. Chem. **101**:685, 1933.
23. Stoddard, J. L., and Drury, P. E.: J. Biol. Chem. **84**:741, 1929.
24. Man, E. B., and Gildea, E. F.: J. Biol. Chem. **99**:43, 1932.
25. Bogdanovitch, S. B., and Man, E. B.: Am. J. Physiol. **122**:73, 1938.
26. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.

The method of extraction used in the present analyses may be subject to criticism because of its relative gentleness as contrasted with long-continued Soxhlet or other continuous extraction methods often employed in the determination of tissue lipids. In a number of instances the completeness of the extraction was checked by subjecting the extracted residue remaining on the filter paper to further extraction by a Soxhlet method involving hot alcohol extraction for twenty-four hours and ether extraction for a subsequent eight hours.[27] Except in 1 instance, no further significant quantities of lipid were obtained by this more vigorous method. In the exception an additional quantity of lipid amounting to 30 per cent of the lipid originally extracted was recovered in the Soxhlet extract; in this instance, however, an unusually large aliquot of tissue (over 5 Gm.) had been inadvertently used. It was noted that the residue of extracted adrenal pulp appeared grossly fatty and gummy on the filter paper and delayed filtration of the alcohol-ether extract for many hours, a phenomenon never again encountered. It was felt that under the conditions described the simple extraction method utilized recovered at least 95 per cent of the total adrenal lipid in all other instances.

A similar comment relates to the method of determining total lipid in the original alcohol-ether extract. In 5 instances, duplicate determinations were made, using the more detailed technic recommended by Bloor[28] of rectifying by redissolving in petroleum ether, separating and reevaporating, prior to weighing. In each case the results agreed within 5 per cent with those obtained by the simpler method, and the rectification technic was therefore not routinely used.

Since the rate at which the quantity of any lipid component may change on storage at room and reduced temperatures was of evident interest in connection with the analyses performed, an attempt was made to assess this factor. Adrenal glands obtained at autopsies of dogs were finely minced, and an aliquot taken for immediate analysis. The remaining minced tissue was reserved for later analysis, a part being permitted to remain at room temperature and a part being kept in a freezing locker at —18 C. The results of such comparative analyses are shown in table 2, in which the values of the various lipid components are expressed as percentages of dry weight. The analytic methods used were identical with those used for the human adrenal glands. Agreement was found to be fairly good, with one prominent exception in the unaccountable discrepancies for dog W152. It is of interest that, among the various components, phospholipid and free cholesterol showed the greatest fluctuations and fatty acids were the most stable under these conditions. It may be noted that keeping minced adrenal tissue at room temperature under nonsterile conditions is perhaps an unreasonably strict test for stability. Similarly, mincing alone may favor the loss of lipid in tissue juice, which is not homogeneously represented even in the frozen samples. If consistent and significant changes did occur under the conditions of storage, however, or in the interval between death and autopsy, they were, it is reasonable to surmise, random ones, in the series of analyses reported, and without influence on comparisons made between groups of results in different clinical categories.

In 64 of the 77 autopsies furnishing adrenal glands for analysis the time of autopsy was accurately recorded, and the average period elapsing between death

27. These analyses were made by Dr. Clara M. Szego, of the department of physiological chemistry of Yale University.

28. Bloor, W. R.: Biochemistry of the Fatty Acids and Their Compounds, the Lipids, New York, Reinhold Publishing Corp., 1943.

and autopsy was approximately seven hours, with a range of from one to eighteen hours. In almost every case, the body was placed in a refrigerated room within two hours after death and kept there until the time of the autopsy.

Calculation of Results.—The basic data derived by chemical analysis were values for total lipid, total titratable fatty acids in milliequivalents, lipid phosphorus, free and total cholesterol. By appropriate calculations, values were derived in grams for fatty acids, phospholipid, ester cholesterol and the fatty acid constituents associated with ester cholesterol and phospholipid, respectively. Neutral fat fatty acid was estimated by subtracting phospholipid and cholesterol fatty acid from total fatty acid. Certain assumptions were made in these calculations. A C_{20} fully saturated fatty acid was taken arbitrarily as the average molecular size and the number of grams of fatty acid calculated from milliequivalents of acid on this basis. Lecithin was considered the model phospholipid in calculating grams of phospholipid from grams of lipid phosphorus.

When such calculations were made, it was possible to determine the total lipid in two independent ways: (1) by using the data derived from actual analysis

TABLE 2.—*Stability of Lipid Fractions in Adrenal Glands of Dogs Kept at Room and Refrigerator Temperatures**

Dog	Condition	Total Lipid	Fatty Acid	Phospho-lipid	Free Choles-terol	Ester Choles-terol
W-1	At autopsy	60.2	28.5	17.6	6.3	21.1
	Room temperature 72 hr.	62.2	28.9	19.8	7.8	20.0
	Freezer 1 week	63.6	31.8	20.3	8.9	21.2
B-53	At autopsy	59.2	29.9	22.9	8.0	19.0
	Room temperature 72 hr.	63.8	31.5	18.5	6.5	19.4
	Freezer 72 hr.	69.3	30.4	21.1	6.2	20.7
W-323	At autopsy	63.3	33.1	18.7	6.1	20.6
	Room temperature 24 hr.	65.9	32.4	13.8	4.5	18.8
	Freezer 1 week	63.0	30.1	16.1	5.6	15.8
W-152	At autopsy	86.5	46.0	31.2	8.5	15.9
	Room temperature 24 hr.	63.2	29.3	17.0	3.2	19.9
	Freezer 1 week	62.9	33.9	17.1	4.3	13.0

* Each value is expressed as percentage of the dry weight of adrenal tissue.

of the glands for total lipid and (2) by adding the various independently determined fractions, after making necessary corrections, such as the subtraction from each value for phospholipid of the proportionate weight of the two fatty-acid molecules present in each molecule of lecithin. It is of interest to note the reasonably good agreement between calculated and determined values of total lipid. The average percentage discrepancy in absolute terms between these two values was 8.3, with a standard deviation of 7.6 for a group of 69 sets of adrenal glands.

Clinical Categories.—Study of the clinical and autopsy records of persons whose adrenal glands were subjected to lipid analysis permitted the construction of five different categories: 1. Normal persons, dead as the result of violence, without evidence of significant disease. 2. Miscellaneous patients, whose deaths were due to a variety of diseases commonly encountered in a general hospital but not to significant infection, hypertension or any marked grade of arteriosclerosis. 3. Persons whose deaths resulted from infections, chiefly severe septic infections such as peritonitis, septicemia, meningitis and bacterial pneumonias. 4. Persons who died with arteriosclerosis, i.e., with marked atherosclerosis involving major vessels, usually those of the heart, the brain and the kidneys. Most deaths in this group were considered the result of coronary insufficiency, with or without

myocardial infarction. Hypertension could be excluded on the basis of adequate clinical data covering months or years prior to death, absence of significant cardiac hypertrophy and absence of renal arteriolosclerosis. 5. Persons who died with long-sustained hypertension, usually at high levels, and associated with marked cardiac hypertrophy and arteriolosclerosis of the kidney and other organs. All the cases selected met the two criteria suggested by Moritz and Oldt[29]: sustained blood pressure levels greater than 150 systolic and 100 diastolic or 160 systolic and 90 diastolic and a heart weight greater than 400 Gm. in males or 350 Gm. in females.

RESULTS

In table 3 are shown the data concerning the lipid components derived from analyses of 77 pairs of adrenal glands under the clinical groupings cited in the previous section. In table 4 these data are summarized in the presentation of the mean values and mean standard errors for total lipid, fatty acid, phospholipid, free and ester cholesterol, each expressed both as percentage of the dry weight of the glands, as total quantity in both adrenal glands and as percentage of total lipid. Confidence limits for significant differences are taken as a p value less than 0.01.

Significant differences of the mean values for the dry weight of both adrenal glands were not detected between any two of the clinical groups. Borderline significance (p less than 0.02) may be attributed to the difference between 2.9 Gm. for the mean adrenal weight in the hypertensive group and 2.1 Gm. for that in the infectious group.

The value of total lipid expressed as percentage of dry weight of adrenal tissue was not significantly different in the hypertensive, the arteriosclerotic and the normal group. In each of these groups, however, there was a significantly greater concentration of lipid in the adrenal glands than in the infectious disease group. Adrenal glands of the hypertensive and the normal group also contained significantly more total lipid than those of the miscellaneous group. In the arteriosclerotic group this value was greater than in the miscellaneous group by an amount of borderline significance (p less than 0.02). The infectious and miscellaneous groups did not differ significantly in their adrenal lipid levels.

Similar relationships are seen on comparing the total quantities of adrenal lipid present in the various groups, with the exception that the small number of values for the normal group (owing to the difficulty of obtaining both entire adrenal glands at the autopsies of normal persons) makes statistical comparison invalid apart from enormous differences.

In terms of percentage of dry weight of adrenal tissue, roughly the same relations hold for fatty acid as for total lipid among the various groups. Adrenal glands of normal, hypertensive and nonhypertensive arteriosclerotic persons were not statistically distinguishable, while those of the miscellaneous group contained a greater concentration of fatty acids than those of the infectious group by a factor of only borderline significance. Greater concentrations of fatty acid were found in the normal, hypertensive and arteriosclerotic groups than in the infectious group.

Examination of the values for total quantity of fatty acid indicates similar relationships, with the exception again that the small number of normal values eliminates this category from statistical consideration because of the high standard error associated with even slight variation in a small population. When fatty acid was considered as percentage of total lipid, an entirely different set of rela-

29. Moritz, A. R., and Oldt, M. R.: Am. J. Path. **13**:679, 1937.

TABLE 3.—*Weights and Lipid Fractions of Human Adrenal Glands**

Sex	Age	Principal Clinical and Autopsy Observations	Wet Wt. Both Adrenal Glands, Gm.	Dry/ Wet Ratio	Dry Wt. Both Adrenal Glands, Gm.	Gm. per 100 Gm. Dry Weight				
						Total Lipid	Fatty Acid	Phospholipid	Free Cholesterol	Ester Cholesterol
		Normal Group								
F	34	Death in automobile accident—transection of spinal cord	4.4	0.41	1.3	63.1	36.3	9.9	7.2	24.5
M	30	Instantaneous death from gunshot wound of chest	5.8	0.36	2.1	62.5	29.1	9.4	6.1	30.0
M	45	Instantaneous death from injury of the head	...	0.41	...	65.4	36.8	8.3	3.0	32.7
M	51	Instantaneous death from injury of the head	7.6	0.31	2.4	53.3	47.7	9.4	3.8	21.9
M	60	Death from fall	12.9	0.42	5.4	64.3	28.3	5.2	9.2	22.4
M	50	Stab wound through heart	...	0.37	...	66.0	33.8	10.4	6.3	31.8
F	24	Found dead with pulmonary embolus; 3 months pregnant	...	0.33	...	58.8	26.2	11.4	6.9	26.4
F	8	Instantaneous death in automobile accident—fractured skull	...	0.38	...	50.7	29.4	16.6	6.3	18.0
M	36	Fractured skull; epilepsy	...	0.24	...	48.9	24.1	12.7	7.7	14.6
M	35	Found dead after fight—probable strangulation	...	0.32	...	58.4	30.9	11.2	9.0	15.9
M	24	Died shortly after stab wound of heart	...	0.43	...	63.9	32.1	8.1	6.2	22.7
M	34	Suicide with carbon monoxide	6.0	0.39	2.3	55.8	26.6	8.1	7.9	20.5
M	21	Instantaneous death from bullet wound of chest	...	0.38	...	62.8	32.0	8.6	6.2	22.7
		Hypertension Group								
M	54	Severe hypertension 10 years; death during sympathectomy	...	0.45	...	59.9	38.9	12.2	1.4	21.1
M	47	Severe hypertension 8 years; terminal renal and cardiac failure	10.3	0.26	2.7	53.0	31.8	14.8	2.5	12.2
F	67	Hypertension 11 years; terminal cardiac failure	10.1	0.35	3.5	63.0	30.4	8.8	1.2	28.2
F	37	Hypertension 3 years; severe diabetes and glomerulosclerosis	7.9	0.30	2.4	42.6	22.4	10.4	2.4	28.0
F	66	Hypertension 2 years; diabetes and chronic pyelonephritis	7.4	0.33	2.4	53.8	....	8.8	2.7	22.4
F	48	Hypertension, cardiac failure 1 year; terminal uremia	9.9	0.28	2.8	61.1	30.7	10.4	3.0	23.3
		Arteriosclerosis Group								
F	65	Diabetes, myocardial infarct	3.9	0.35	1.4	55.0	35.2	12.0	1.3	15.6
F	54	Myocardial infarct, terminal uremia	6.6	0.32	2.1	57.2	30.9	11.7	3.9	13.2
M	77	Acute congestive failure, large myocardial scars	7.4	0.37	2.7	65.5	31.8	7.5	6.1	26.3
M	57	Myocardial infarct	6.2	0.40	2.5	64.6	33.8	13.3	3.6	24.7
F	60	Coronary occlusion without infarct, mild diabetes	5.2	0.33	1.7	52.0	26.0	12.7	4.5	17.1
M	56	Coronary occlusion without infarct	6.4	0.37	2.4	61.8	28.2	8.8	7.6	24.3
		Miscellaneous Group								
F	36	Glioblastoma cerebrum; death 1 day after craniotomy	7.8	0.35	2.7	51.0	25.6	12.2	2.3	17.0
M	59	Chronic prostatitis, cystitis, pyelonephritis, uremia	7.4	0.39	2.9	65.2	....	7.5	1.1	19.3
F	15	Acute polioencephalomyelitis; death from respiratory paralysis	7.7	0.26	2.0	34.4	20.7	15.6	1.9	9.2
F	27	Death in diabetic coma	8.7	0.28	2.4	36.4	21.3	14.6	2.8	18.6
M	41	Portal cirrhosis	5.5	0.20	1.1	51.7	32.4	15.6	3.7	11.2
M	54	Syphilitic aortitis, stenosis of coronary ostiums, cardiac failure	5.8	0.31	1.8	47.3	26.3	11.7	4.3	10.2
		Infection Group								
M	77	Pneumococcic lobar pneumonia	...	0.34	...	43.0	26.1	8.3	1.6	5.0
F	55	Empyema of gallbladder with peritonitis	8.4	0.25	2.1	47.9	26.6	13.0	1.6	9.5
F	80	Lobar pneumonia	5.7	0.27	1.5	32.8	23.6	14.0	1.0	11.4
F	33	Perforation of uterus and peritonitis post partum	8.4	0.32	2.7	24.7	....	....	11.9	0.8
M	57	Acute pyelonephritis, hypertension, uremia	10.9	0.25	2.7	41.5	18.7	14.0	4.6	9.2
M	3	Meningococcemia, Waterhouse-Friderichsen syndrome	5.8	0.32	1.9	23.2	15.6	8.1	5.9	5.1

* Values for all the members of the normal group and for 6 representative persons in each of the other groups are shown. The total number of persons whose adrenal lipid values were determined included 19 with hypertension, 15 with arteriosclerosis without hypertension, 18 with miscellaneous disorders and 12 with septic infections.

tionships appeared: Now the highest values were found in the groups of persons dying of infectious and miscellaneous diseases—statistically indistinguishable from each other—and both significantly different from the remaining three groups.

With phospholipid expressed as percentage of dry weight of adrenal tissue, the notable feature of a comparison of any two of the five groups was the relative constancy of the values for phospholipid, there being no significant differences between any of the groups. Not only mean values but individual values as well (see table 3) showed relatively great consistency as compared with the other fractions measured.

Expressed as percentage of total lipid, phospholipid followed the same pattern in the various groups as did fatty acid, being greatest in the miscellaneous and infectious disease group and smallest in the arteriosclerotic, hypertensive and normal categories.

TABLE 4.—*Mean Values of Adrenal Lipid Fractions in Various Clinical Categories* *

	Normal	Hypertension	Infection	Arteriosclerosis	Miscellaneous
Dry weight of adrenal tissue..	2.8 ± 0.66 (5)	2.9 ± 0.23 (18)	2.1 ± 0.18 (11)	2.6 ± 0.24 (14)	2.3 ± 0.19 (14)
Total lipid, % dry weight.....	60.3 ± 1.94 (13)	57.7 ± 1.51 (19)	37.0 ± 3.32 (11)	55.9 ± 2.44 (15)	45.9 ± 3.13 (17)
Total lipid, total amount.....	1.7 ± 0.79 (5)	1.7 ± 0.14 (18)	0.8 ± 0.10 (10)	1.5 ± 0.09 (14)	1.1 ± 0.12 (14)
Fatty acid, % dry weight.....	30.1 ± 1.12 (13)	29.0 ± 0.99 (18)	21.4 ± 1.28 (11)	29.9 ± 1.09 (15)	26.4 ± 1.45 (16)
Fatty acid, total amount.....	0.8 ± 0.17 (5)	0.9 ± 0.07 (17)	0.4 ± 0.04 (10)	0.8 ± 0.08 (14)	0.6 ± 0.05 (13)
Fatty acid, % total lipid.....	49.7 ± 1.12 (13)	50.4 ± 1.31 (18)	56.9 ± 2.83 (10)	54.0 ± 1.72 (15)	58.4 ± 2.48 (15)
Phospholipid, % dry weight..	9.9 ± 0.76 (13)	10.2 ± 0.57 (19)	11.9 ± 0.87 (10)	11.0 ± 0.60 (14)	12.6 ± 1.90 (15)
Phospholipid, total amount...	0.21 ± 0.017 (5)	0.28 ± 0.016 (18)	0.29 ± 0.036 (9)	0.26 ± 0.020 (13)	0.28 ± 0.022 (14)
Phospholipid, % total lipid...	16.7 ± 1.5 (13)	18.1 ± 1.35 (19)	33.0 ± 4.0 (9)	20.9 ± 2.0 (14)	28.0 ± 2.9 (15)
Free cholesterol, % dry weight	6.6 ± 0.49 (13)	4.9 ± 0.59 (19)	3.4 ± 0.94 (12)	3.4 ± 0.47 (15)	3.3 ± 0.58 (17)
Free cholesterol, total amount	0.20 ± 0.078 (5)	0.15 ± 0.022 (18)	0.08 ± 0.026 (11)	0.09 ± 0.018 (14)	0.07 ± 0.019 (14)
Free cholesterol, % total lipid	11.2 ± 0.89 (13)	8.5 ± 0.95 (19)	11.1 ± 4.2 (11)	5.9 ± 0.7 (15)	7.1 ± 1.19 (16)
Ester cholesterol, % dry weight	23.2 ± 1.62 (13)	21.5 ± 1.27 (19)	7.9 ± 2.04 (12)	20.5 ± 2.06 (15)	12.9 ± 1.86 (17)
Ester cholesterol, total amount	0.70 ± 0.14 (5)	0.65 ± 0.063 (18)	0.32 ± 0.097 (11)	0.57 ± 0.056 (14)	0.36 ± 0.20 (14)
Ester cholesterol, % total lipid	38.2 ± 2.14 (13)	37.5 ± 2.35 (19)	18.3 ± 4.09 (11)	36.5 ± 3.88 (15)	28.1 ± 3.75 (16)

* Each of the values shown is the mean and the standard error. The number in parentheses is the number of persons in the group. All values not percentages are in grams.

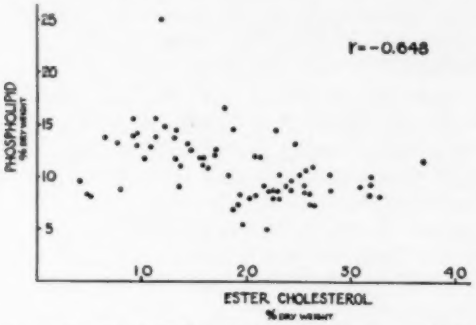
The concentration of free cholesterol per unit of dry weight was found to be highest in the normal group, less high (although not significantly so) in the hypertensive group and relatively consistently low in the remaining three groups. In considering the total amount of free cholesterol present in both adrenal glands it is of interest to note the absence of significant differences except between the hypertensive and the miscellaneous group. Expressed as percentage of total lipid, free cholesterol was highest in the normal group, lowest in the arteriosclerotic group—between which two values the only difference of significance was detected.

Ester cholesterol expressed as percentage of dry weight of adrenal tissue showed considerable variation among the individuals of certain groups, notably the infectious disease and miscellaneous groups, as may be observed by inspecting the relative magnitude of the standard error of the mean. Despite large intragroup variations, however, the difference between certain of the group means was so great as to permit an appearance of significance. As with total lipid, the five clinical categories fell into two larger groups, one of relatively high ester cholesterol content, comprising arteriosclerotic, hypertensive and normal persons, the other with low ester cholesterol content, comprising the persons whose deaths were ascribed to

miscellaneous diseases or to infectious diseases but who were neither hypertensive nor arteriosclerotic. Significant differences did not appear between the individual categories within each of these two larger groups.

Values for the total quantity of ester cholesterol in both adrenal glands were statistically distinguishable only on comparing the hypertensive and the infectious disease group. Expressed as percentage of total lipid, however, ester cholesterol was significantly higher both in normal and in hypertensive persons than in persons in the infectious or miscellaneous disease groups.

Relatively consistent values, both for individuals and for the clinical groups, were observed for two constituents: fatty acids, expressed as percentage of dry weight of adrenal tissue (range of variation from 21.4 for the infectious disease group to 30.1 for the normal group) and expressed as percentage of total lipid (range of variation from 49.7 for the normal group to 58.4 for the miscellaneous disease group); and phospholipid, expressed as percentage of dry weight of adrenal tissue (ranging from 9.9 for the normal group to 12.6 for the miscellaneous disease group) and expressed as total quantity (ranging from 0.21 Gm. for the



Relation between the phospholipid and ester cholesterol concentrations in human adrenal glands.

normal group to 0.29 Gm. for the infectious disease group). Not only the limited range of mean values but also the relatively small standard errors distinguished these components in their consistent levels.

An additional point of interest is suggested by the reciprocal relationship between phospholipid and ester cholesterol manifest in table 4: The groups with the highest ester cholesterol values (normal, hypertensive and arteriosclerotic persons) had, associated with these values, the lowest phospholipid values, whether these were expressed as percentage of dry weight of adrenal tissue, as total quantity present or as percentage of total lipid. Further investigation of this relationship revealed that a significant inverse correlation existed between ester cholesterol and phospholipid (both expressed as percentage of dry weight) when the two values were compared in each pair of adrenal glands analyzed. The correlation coefficient, r, was calculated to be —0.648, and was associated with a p value much less than 0.01 (figure).

COMMENT

The results reported in this study indicate that several lipid components of adrenal glands (total lipid, ester cholesterol, fatty acids)

are not present in concentrations higher than normal in persons with significant hypertension or arteriosclerosis, a finding which may also be inferred from previously reported data. It seems equally well established that a group of patients with atherosclerosis but free of hypertension would be virtually indistinguishable from a group of hypertensive persons with respect to adrenal lipids. There is a greater or less degree of depletion of these lipids in the adrenal glands of persons dying with a variety of other diseases, most notably infections.

The impression of many previous investigators that supernormal lipid levels are found in the adrenal glands of hypertensive patients seems to have been based on the unjustified use of a group of non-hypertensive persons with miscellaneous diseases as a reference group with which to compare the hypertensive group in judging adrenal lipid values. The data presented in the present report support the conclusion that adrenal lipids are not abnormally elevated in instances of hypertensive and arteriosclerotic disease, but remain at normal levels not maintained in other disorders.

A purely descriptive correlative study like the present one involves certain inherent difficulties of interpretation, further complicated by the use of discontinuous categories in which to classify a continuum of data. First, it may be recognized that clinical groupings of the sort made are meaningful for nosologic purposes but may actually conceal significant physiologic factors causally associated with changes in tissue lipids. Thus, the factors determining the levels of adrenal lipid fractions may be unconsciously selected correlates of the clinical groups chosen.

In a descriptive study, hundreds of possible correlates of the classifications made suggest themselves, and can be eliminated only by a number of analyses sufficient to permit isolated statistical evaluation of every conceivable contributory factor. A few such possibilities are: 1. Presence of associated metabolic disturbances, such as diabetes, which occurred in a considerable number of the hypertensive group. 2. Occurrence of renal failure, certainly a much commoner terminal event in hypertensive and arteriosclerotic disease than in the other clinical groups. The observations already referred to, demonstrating an increase in the lipid content of adrenal and other tissues following nephrectomy and ureteral ligation, make this possibility more than a remote one. 3. General nutritional state, which might be more influenced by infection than by cardiovascular or renal disease.

Isolation and separate analysis of these factors are not possible in a study embracing relatively few analyses. A few simple factors, however, were subject to estimation. Sex could be eliminated, since in all but the normal group there was no preponderant sexual selection

of cases, the numbers of men and women being approximately equal in the hypertensive and the infectious disease group. Age did not seem to be a factor, although the mean for the miscellaneous group was relatively low (37 as contrasted with 51, 63 and 64 for the infectious disease, arteriosclerosis and hypertension groups, respectively); all ages were represented in each group. Duration of illness and hospitalization may be considered a factor of possible importance: If adrenal cholesterol is reduced following stresses of wide variety, duration of disease might play a role in determining the level of adrenal cortical lipid at death. Furthermore, the arteriosclerotic group might be suspected to include a large proportion of deaths following brief and sudden illnesses, since both myocardial infarction and cerebral hemorrhage were common events in this group. Examination of this factor, however, did not reveal any prominent difference between mean numbers of hospital days for any two groups, the values being 9.6, 11.8, 17.5 and 13.7 for the groups of patients with arteriosclerosis, infections, miscellaneous diseases and hypertension, respectively. A considerable range was present in each group.

In any event, lengthy speculation as to the possible biologic or pathologic significance of the results determined seems supererogatory. The depletion of adrenal cholesterol and, as appears from the present study, fatty acids, in infectious diseases has already been clarified by experimental work relating this phenomenon to an increased rate of secretion of corticosteroid substances. The less prominent, but still distinct, reduction of the same lipids in the many other disease states may tentatively be considered a similar response of the pituitary–adrenal cortical system to undefined stimuli incident to a variety of metabolic disturbances.

The unique maintenance of adrenal cortical lipids at normal levels in persons with hypertensive and arteriosclerotic disease offers another facet of similarity in these two groups. Whether a single common factor operative in both groups is responsible, or whether a fortuitously similar state results from independent mechanisms cannot be discerned. Disturbances of cholesterol metabolism, which are of current interest in the experimental investigation of atherosclerosis, may be related to the preservation of relatively high adrenal cholesterol levels in the arteriosclerotic patient. Similarly, proper interpretation of the comparable state of adrenal lipids in the hypertensive person may take its place in the present fragmentary array of evidence relating hypertension to the state of adrenal cortical function.[31]

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30. MacKay and MacKay.[17] Winkler and others.[18]

31. Perera, G. A.: J. A. M. A. **129**:537, 1945. Shorr, E.: Am. J. Med. **5**:783, 1948.

A final logical solecism must be admitted. The foregoing discussion is predicated on the assumption that in hypertension and arteriosclerosis, adrenal lipid levels are defended against stimuli common to all disorders, which would otherwise lower them by the time of death. It seems perhaps more justified to conclude that preservation of these levels results simply from absence of adequate stimuli of the sort present in infectious and other nonvascular diseases. Also, since the level of any given tissue component is the resultant of several rates, involving production, utilization and transport, it cannot be said whether preservation of normal levels of lipid in the instances noted may not reflect altered, though balanced, rates of accumulation and removal.

SUMMARY

Analyses of human adrenal glands to determine the levels of the major lipid components were conducted on material derived from 77 autopsies, and the results were compared in several clinical classifications. A group of persons dying as the result of violence furnished material for the description of normal data, represented in the literature poorly or not at all. Certain lipid fractions, notably ester cholesterol, were present in similar concentrations in hypertension and arteriosclerosis, being maintained at levels comparable to those found in adrenal glands of persons dying without disease. In infections and in most disorders other than those characterized by marked hypertension or arteriosclerosis, the concentration and the total quantity of these components were markedly diminished. The quantity of phospholipid, like that of fatty acid, tended to be quite constant both in individuals and in the various clinical groups, for which averaged values were recorded. Phospholipid expressed as a percentage of the dry weight of adrenal tissue varied inversely with total lipid and ester cholesterol, being highest in the infectious disease and miscellaneous disease groups and lowest in the hypertensive and normal groups. This relationship was even clearer when phospholipid was expressed as a percentage of the total lipid present.

ACTION OF IODINE ON GOITERS PREVIOUSLY TREATED WITH THIOURACIL

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THE THERAPEUTIC use of substances, such as thiouracil, which have a powerful functional and morphologic effect on the thyroid gland has introduced a certain confusion into the anatomoclinical interpretation of goiter, which until 1943 (Buño [1]) rested on a firm base.

Hyperplasia of the thyroid gland (very large in some cases) was found experimentally, which was characterized by histologic signs of hyperactivity and yet was accompanied by hypothyroidism. The mechanism of this apparently paradoxic and contradictory behavior was made clear by further experimental work.

At the Massachusetts General Hospital, under the direction of Means and co-workers [2] comparisons were made between specimens of the thyroid gland obtained prior to thiouracil treatment and the gland removed after such treatment. Following administration of thiouracil, a slight increase of the average height of cells had been observed, also general proliferation and hyperplasia of the gland with increased vascularization and in some instances hyperplasia of interstitial lymphocytic tissue. In 7 patients treated by them, first with thiouracil alone and then with thiouracil and iodine together, the initial increase in average cell height obtained with the first therapy was followed by a decrease when iodine was given. Although the authors have not given a histologic description of their preparations, their photomicrographs indicate that the iodine administered with the thiouracil not only modified the height of cells but also the amount of stored colloid. The gland showed a medium degree of activity with numerous absorption vacuoles.

From the Department of Histology and Embryology, Montevideo Faculty of Medicine (Dr. Buño) and the Institute of Endocrinology (Dr. Grosso).

1. Buño, W.: Estudios de histofisiología e histopathología tiroidea, Buenos Aires, El Ateneo, 1943.

2. Rawson, R. W.; Evans, R. D.; Means, J. H.; Peacock, W. C.; Lerman, J., and Cortell, R. E.: J. Clin. Endocrinol. **4**:1, 1944. Rawson, R. W.; Moore, F. D.; Peacock, W. C.; Means, J. H.; Cope, O., and Riddell, C. B.: J. Clin. Investigation **24**:869, 1945.

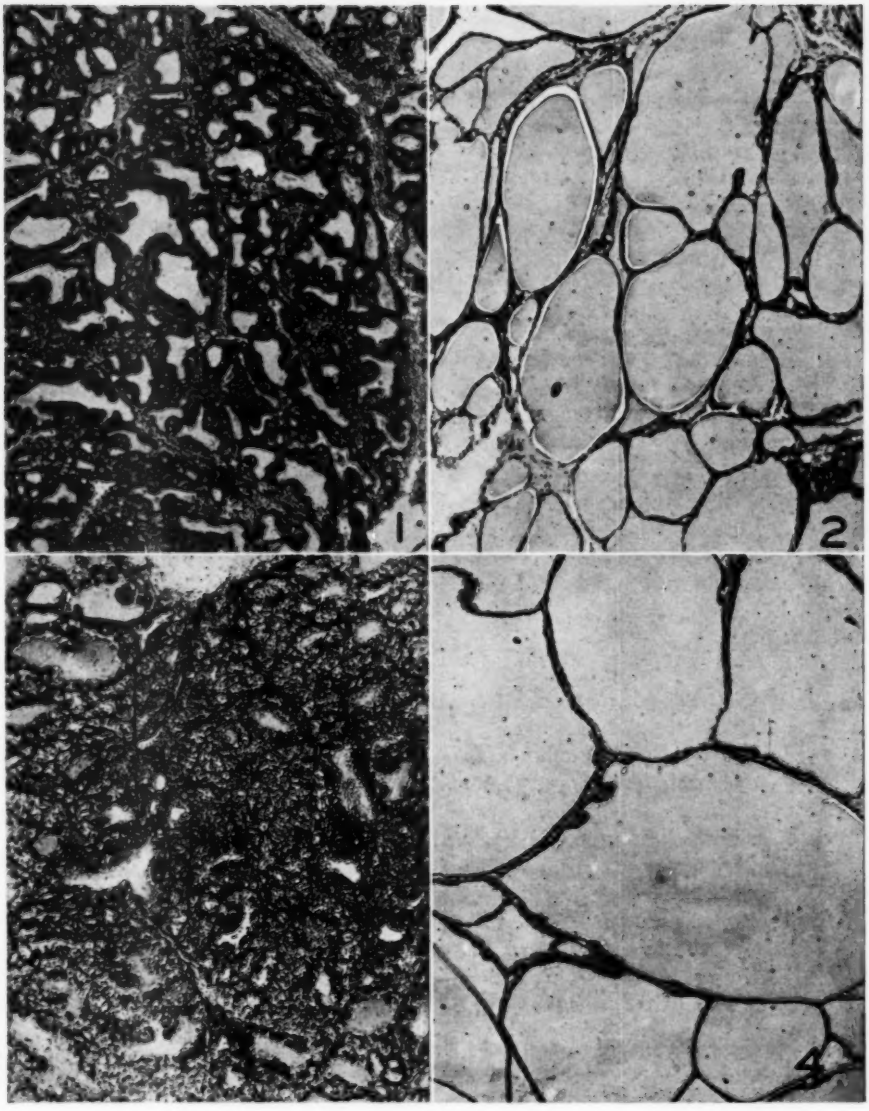

Fig. 1.—Lobe of the thyroid gland removed after the patient had been treated solely with thiouracil (× 420; basal metabolic rate, + 7).

Fig. 2.—Second lobe removed from the same patient four months after that in figure 1. The preoperative treatment was solely with iodine. (× 420; basal metabolic rate, + 35.)

Fig. 3.—Lobe removed after the patient had been treated solely with thiouracil. (× 420; basal metabolic rate, + 15.)

Fig. 4.—Second lobe removed from the same patient as that in figure 3 four months later. The preoperative treatment was solely with iodine. (× 420; basal metabolic rate, + 42.)

Halpert, Cavanaugh and Keltz [3] made a histopathologic study of thyroid glands of patients with exophthalmic goiter, 2 of whom had been treated solely with thiouracil, 3 with iodine followed by thiouracil and 2 with thiouracil first, then with thiouracil and iodine combined. The last two interest us most: One of them had received iodine four months before starting to take thiouracil, and in the course of treatment given before a second operation, was treated with thiouracil and iodine combined, all of which makes the interpretation of the case complicated. The other of these 2 patients was operated on in one stage, so that there is no proof as to what the state of the gland was before the iodine treatment, and, besides, the iodine was given together with thiouracil.

The thyroid glands studied by us, from patients with exophthalmic goiter [4] who prior to operation had been treated with thiouracil, showed the characteristic appearance of intense hypersecretion, even when the metabolism was normal or below normal and the patient presented signs of hypothyroidism. But we particularly wish to draw attention to 2 cases in which thyroidectomy was carried out in two stages with a four month interval between the operations.

Thiouracil had been given prior to the first operation, and the histologic appearance was that of the hyperplasia and hyperactivity known to be produced by this drug (figs. 1 and 3).

On the other hand, prior to the second operation iodine alone was used in both patients, and the histologic picture obtained was remarkably different from that of the lobe removed four months earlier (figs. 2 and 4). The thyroid follicles were enlarged, lined with low epithelium, and contained abundant dense colloid; in fact, the picture corresponded to that of colloid goiter. At some points, however, there were still to be seen a few small follicles with epithelium of medium height.

The contrast between these two types of structure can be seen by comparing the corresponding photomicrographs (for all sections the magnification is the same).

COMMENT

The administration of thiouracil produces a marked reduction of the iodine content of the normal thyroid gland (Astwood and Bissell [5]; Franklin, Chaihoff and Lerner [6]). The gland depleted of iodine can again store large quantities of it when iodine is administered intravenously after suspension of thiouracil treatment (Astwood [7]).

3. Halpert, B.; Cavanaugh, J. W., and Keltz, B. F.: Arch. Path. **41**:155, 1946.

4. The material was obtained from the Institute of Endocrinology. The clinical study was made by Assistant Prof. J. M. Cerviño; the operation was done by Dr. G. Caprio.

5. Astwood, E. B., and Bissell, A.: Endocrinology **34**:282, 1944.

6. Franklin, A. L.; Chaihoff, I. L., and Lerner, S. R.: J. Biol. Chem. **153**:265, 1944.

7. Astwood, E. B., in The Harvey Lectures, 1944-1945, Lancaster, Pa., Science Press Printing Company, 1945, vol. 40, p. 195.

The experiments of VanderLaan and Bissell,[8] in which iodine and thiouracil were given simultaneously, seem to indicate that the gland can in reality metabolize large quantities of iodine but is totally incapable of storing it. Hence, the thyroid tissue does not contain iodine in any appreciable quantity if this is determined a few hours after administration of the element. On the other hand, thirty minutes after an injection, the content of iodine is considerable.

Mackenzie,[9] administering combinations of goitrogenic substances with adequate quantities of sodium iodide, was able to prove that iodine completely inhibits the hyperplastic reaction produced in the thyroid gland by thiouracil. On the other hand, it is interesting to note that it increases the hyperplastic reaction produced by sulfaguanidine.

Our observations seem to indicate that likewise in patients with exophthalmic goiter thiouracil increases thyroid avidity for iodine. As has been shown by many observations, iodine alone is not capable of producing in the thyroid gland of a patient with exophthalmic goiter that state of colloid transformation which is reached when iodine is given after the administration of thiouracil. This would agree with experiments of Larson, Keating, Peacock and Rawson,[10] who demonstrated in the chicken that when the administration of thiouracil is stopped the capacity of the thyroid gland to store radioactive iodine increases greatly.

Besides, the histologic appearance contrasts with the clinical picture, for in both cases at the moment of the first stage of operation the basal metabolism was lower than at the second stage, while the histologic appearance seemed to indicate the contrary (basal metabolic rate + 7 before and + 35 afterward in one case and + 15 before and + 42 afterward in the other).

SUMMARY

Two thyroid glands have been studied in lobectomy in two stages in patients with exophthalmic goiter. Before the first operation treatment was with thiouracil alone; before the second, four months later, iodine alone was given. Sections from lobes removed at these two operations, when compared, showed a remarkable colloid transformation of the gland.

This seems to justify the conclusion that iodine has a far greater effect on the thyroid gland if the patient has previously been treated with thiouracil and this treatment has been suspended; likewise, that the effect thus obtained is much greater than that obtained exclusively with iodine.

8. VanderLaan, W. P., and Bissell, A.: Endocrinology **39**:157, 1946.
9. Mackenzie, C. G.: Endocrinology **40**:137, 1947.
10. Larson, R. A.; Keating, F. R., Jr.; Peacock, W., and Rawson, R. W.: Endocrinology **36**:149, 1945.

DEPOSITION AND FATE OF PLUTONIUM, URANIUM AND THEIR FISSION PRODUCTS INHALED AS AEROSOLS BY RATS AND MAN

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THE MANNER in which foreign materials are eliminated from the lungs has long been of interest. Robertson [1] has summarized previous investigations. In general, it has been shown that particles inhaled into the lungs may be eliminated in two ways. In both mechanisms the particles are engulfed by phagocytes. Following this, some particles, such as silica, are carried into the lymphatic system of the lung by phagocytes migrating through the alveolar lining.[2] Concerning the other mechanism of elimination it is postulated that particles are transported as far as the ciliated epithelium of the respiratory bronchioles, where they are swept out by ciliary action. The cells responsible for transporting them to the respiratory bronchioles have been identified as dust or septal cells. Fried [3] expressed the belief that they are mesenchymal in origin.

McCutcheon's [4] studies of chemotaxis have shown that the phagocytes of the lung are specialized with respect to the kind of particle which they will engulf. He listed polymorphonucear cells as being positively chemotactic to collodion particles, bacteria, and tissue proteins, negatively chemotactic to aluminum silicate and indifferent to carbon particles. In this respect they are in contrast to dust or septal cells, which phagocytose carbon. Dust cells and monocytes do not display positive chemotaxis but accomplish their work by means of random locomotion.

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1. Robertson, O. H.: Physiol. Rev. **21**:112, 1941.

2. Cole, L. G., and Cole, W. G: Pneumoconiosis (Silicosis): The Story of Dusty Lungs; a Preliminary Report, New York, John B. Pierce Foundation, 1940.

3. Fried, B. M.: Arch. Path. **17**:76, 1934.

4. McCutcheon, M.: Arch. Path. **34**:167, 1942.

The hazards of various industrial materials in the lungs have been of concern for many years. Pulmonary elimination and/or absorption of materials became a matter of even greater concern with the release of nuclear energy and the attendant possibility of the accidental release of the products of nuclear fission. However, the production of fission products and fissionable materials had its compensations as far as radiation hazards were concerned, in that the materials which were possible sources of danger because of their radioactivity were by the same token useful tools in the study of the function of the lungs.

Of the several technics available for studying the transport of radioactive materials in the body two were used in these studies. One was the quantitative determination of the behavior of the radioactive materials administered by observing the fate of a known amount of active material introduced into the lungs. This was done by measuring the radioactivity of the animal tissues and excreta with a Geiger-Müller counter in the detection of beta and gamma radiation and the parallel plate linear amplifier combination in that of alpha particles. The second was the determination of the exact location of the particles in lung tissue. This can be done through the use of the technic of radioautography. Radioautographs were taken of lung sections at several periods of time after the lungs had been exposed to the radioactive aerosols used in these studies.

The information gained from these two methods places the investigation on a quantitative basis. Since the materials can always be followed because of their radioactivity, it is possible to determine the various fractions following any particular route as well as the time it takes for translocation to occur.

The elements used were administered as smokes and sprays. The smokes and sprays were composed of particles of plutonium, uranium and the fission products of both of these materials. The mixture of radioactive fission products contained principally strontium (Sr), yttrium (Y), zirconium (Zr), columbium (Cb), ruthenium (Ru), barium (Ba), lanthanum (La), cerium (Ce), cesium (Cs), praseodymium (Pr), neodymium (Nd) and element 61 at the time of the assay of radioactivity in organs, liver, and excreta. Zirconium and protoactinium (Pa) were studied separately as well.

METHODS OF STUDY

The manner in which radioactive material is assayed when present in biologic tissues has been adequately described elsewhere.[5]

5. (*a*) Hamilton, J. G.: The Metabolism of Carrier-Free Fission Products in the Rat, MDDC 1275, June 1946. Technical Information Division, U. S. Atomic Energy Commission, Oak Ridge, Tennessee. (*b*) Scott, K. G.; Axelrod, D. J.; Crowley, J.; Lanz, H., and Hamilton, J. G.: Studies on the Inhalation of Fissionable Materials and Fission Products and Their Subsequent Fate in Rats and Man, MDDC 1276, October 1946. (*c*) Hamilton, J. G.: Radiology **49**:325 and 343, 1947.

The methods which were used to deposit radioactive aerosols in the lungs of rats are as follows: With the exception of the one study of a human subject, young rats weighing between 200 and 300 Gm. were used in all experiments. The rats were prepared for exposure with an intraperitoneal injection of pentobarbital sodium. The dose was 40 mg. per kilogram of body weight for male rats and 30 mg. per kilogram for female rats. After the rat was anesthetized, the whiskers on the nose were closely clipped. The lips were fastened together with a three-cornered stitch between the lower lip and the two halves of the upper lip. The mouth was further sealed up with celloidin (a concentrated pyroxylin), and at the same time the glass nose piece was cemented in place (fig. 1). This procedure completely sealed off the openings of the mouth and directed the rat's respiration through the nasopharynx. In order to complete the exposure, the glass nose piece of the rat was plugged into the aerosol chamber with gum rubber tubing (figs. 2

Fig. 1.—A rat prepared for exposure to aerosol, with nose piece in place and mouth sealed with celloidin.

and 3). After being exposed to the aerosol under study, the rats were placed in metabolism cages so that fecal and urinary excretions could be collected separately. The animals were killed at varying times after exposure, and their tissues, urine and feces were assayed for the element being studied.

The animals used in these studies were given the following materials as aerosols.

1. Plutonium oxide (PuO_2) produced by burning both the metal and the salts of plutonium.

2. Plutonium nitrate ($PuO_2(NO_3)_2$) as a spray.

3. Fission products combined with their parental elements, plutonium and uranium, which had been previously exposed to intense and prolonged neutron

irradiation. Both the plutonium and the uranium were burned in the metallic state, thus producing aerosols of their oxides and the oxides of most of the fission products.

4. Carrier-free fission products, free of uranium and plutonium and containing very small amounts of inactive isotopic elements. They are described as being carrier free because of the fact that they were prepared without inclusion of their stable isotopes during their chemical separation from uranium and plutonium. Most of these also were primarily in the form of their oxides.

5. Aerosols of carrier-free zirconium or zirconium oxide (ZrO_2).

6. Aerosols of protoactinium as protoactinium oxide (Pa_2O_5).

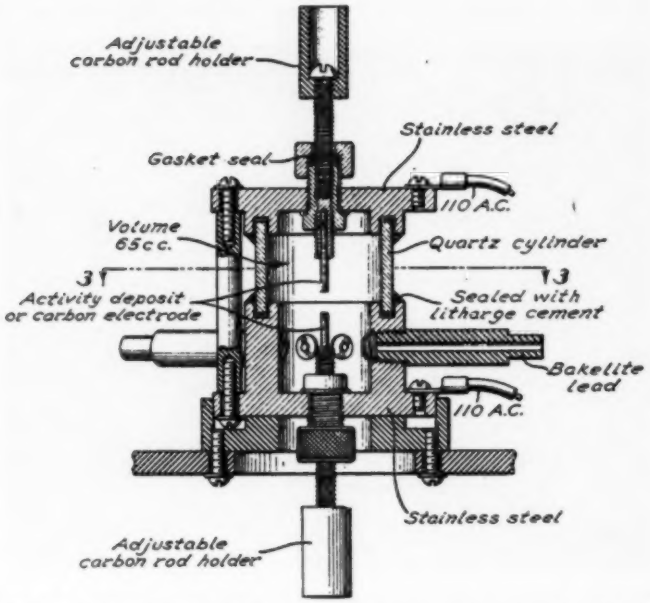

Fig. 2.—The exposure chamber used to produce aerosols by burning the radio-element, deposited on graphite electrodes, in an electric arc. This was done in an atmosphere of pure oxygen in order to prevent the formation of poisonous amounts of carbon monoxide. The bakelite leads were connected to the rat's nose piece with gum rubber tubing. The exposure was made by striking an electric arc between the two electrodes. The exposure time was about 30 seconds.

The specific aerosols used in these studies were prepared as follows:

1. Plutonium nitrate as $PuO_2(NO_3)_2$ is soluble in ethyl ether. Advantage was taken of this property by introducing the plutonium nitrate into the exposure chamber as a spray. Very small particles free from ether were produced by forcing the ether-plutonium solution through a gold jet after it had been allowed to come into equilibrium with carbon dioxide at a pressure of 760 pounds per square inch

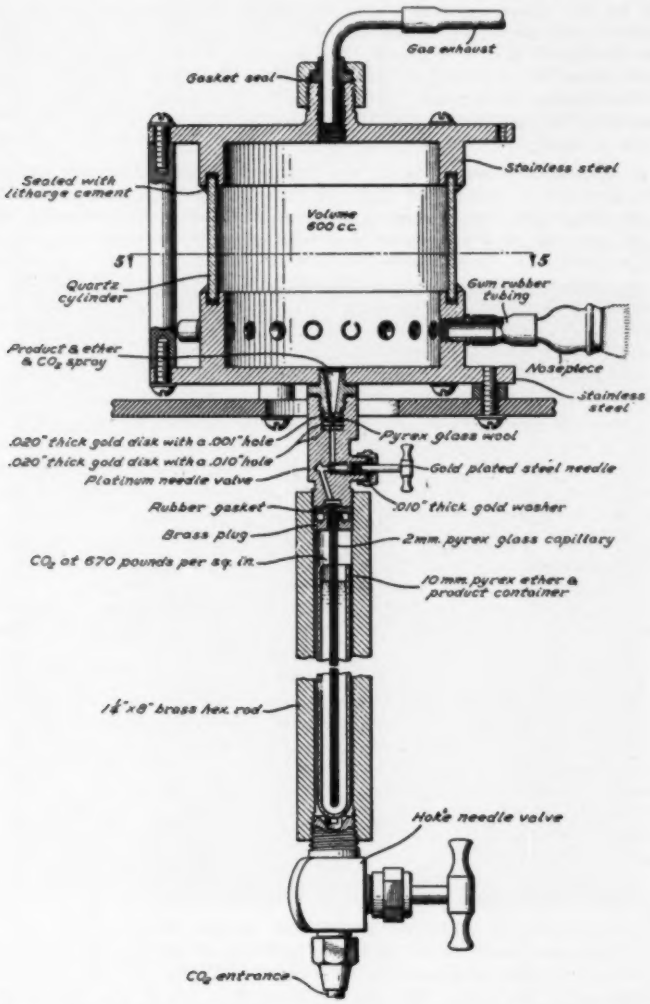

Fig. 3.—Diagrammatic sketch of the equipment used to produce aerosols of plutonium nitrate ($PuO_2[NO_3]_3$). The plutonium nitrate dissolved in ether, with carbon dioxide in the lower chamber, is forced through the gold jet at 760 pounds of pressure into the upper exposure chamber. As the ether evaporates in the warm chamber, the plutonium nitrate aerosol is suspended. Its droplet size is further reduced by the extreme effervescence of the carbon dioxide in the ether at atmospheric pressure.

The drawings in figures 2 and 3 were made by Mr. Gaines Heim.

(53 Kg. per square centimeter). An effervescent ether-plutonium solution was produced when the jet was directed into the warm exposure chamber, the temperature causing the ether to evaporate. The plutonium yielded to the rat was about 10 per cent of the total plutonium used in the spray. The latter amounted to about 300 micrograms of plutonium per exposure. The exposure time was 3 to 4 minutes. The equipment devised to produce the plutonium nitrate aerosols is shown in figure 3.

2. Aerosols of plutonium in the plus four state as plutonium oxide (PuO_2) were produced by burning the plutonium salts and the plutonium metal itself. In the former instance, the solution was evaporated on graphite electrodes, and in the latter the metal was embedded in holes drilled in the centers of the 1/16 inch graphite rods. In both cases the electrodes plus the plutonium were burned in an atmosphere of oxygen in an electric arc. Two hundred micrograms of plutonium were burned for each exposure. The amounts yielded to the rats varied from 4 to 7 per cent of the material burned. The exposure consumed 30 to 60 seconds. One type of equipment used is shown in figure 4.

3. Aerosols of fission products occluded in uranium as uranium oxide (U_3O_8) and plutonium as plutonium oxide (PuO_2) were produced in a similar manner. At the time of exposure the radiations from the fission products predominated. The ratio of fission product disintegrations to the alpha particles arising from the uranium was about 20,000 to 1. The age of the fission products was 2 to 3 months. About 80 mg. of uranium metal was burned at each exposure of 30 seconds' duration. The yield to the rats was about 1 per cent.

4. Carrier-free fission products were also prepared as an aerosol by evaporating the uranium-free solution on graphite rods and burning these. The solution of fission products used was 6 months to 1 year old. Owing to this fact, the fission products with short half-lives had decayed away. The fission elements remaining then were composed primarily of Sr^{89} and Sr^{90}, Y^{91}, Zr^{95}, Cb^{95}, Ru^{106} and Ru^{103}, Cs^{138}, Ce^{144}, and element 61^{147}.

5. Zr^{89} was used in a study of a human subject (one of us). The short half-life of this material, as well as the absence of long-lived radioisotopes, precluded any danger of excessive amounts of radiation to the subject. The active material was suspended as an aerosol in argon as follows: The Zr^{89} solution was evaporated on two concave gold buttons 1 cm. in diameter. It was then dispersed in argon by passing 15,000 volts between two electrodes which were placed 5 mm. apart. A 0.01 microfared condenser was connected across the high voltage line. Oxygen was avoided in the chamber because of the production of undesirable amounts of ozone. Approximately 1 microcurie of Zr^{89} was inhaled by placing in the left nostril a short rubber tube which was also connected to the generator. The inhaled Zr^{89} was exhaled through the mouth into a glass wool filter, followed by several breaths of inactive air, so that the percentage of material exhaled could be calculated.

6. Carrier-free protoactinium, which has a 30 day half-life, was prepared as an aerosol by sparking evaporated Pa^{233} solution on a gold electrode in a high voltage electric discharge. This was administered to rats in the same manner as Zr^{89}.

Except in the Zr^{89} and protoactinium studies, the size of the particles produced by the aerosol generators was determined by collecting representative samples on amyl acetate films. The particle size was estimated with the aid of an electron microscope.

The lungs, kidneys, liver, spleen, skeleton, gastrointestinal tract, head, skin and the balance of the rat, as well as the separated excreta, were assayed for the radioactive material which was administered either as an aerosol or included

with the aerosol. These assays were performed on groups of 3 to 7 rats at periods as long as 256 days after exposure. A detailed report of the amounts of the active material deposited in various tissues of the rat as well as the metabolism of these elements is presented elsewhere.[5] Only values obtained for the head, lung and skeletal deposition, the daily rate of excretion, the particle size and the radioautographic studies are included in this report.

RADIOAUTOGRAPHIC TECHNIC

At intervals, as stated in the text, following the inhalation of the aerosol being studied, the rats were killed with chloroform. The lungs were fixed while intact

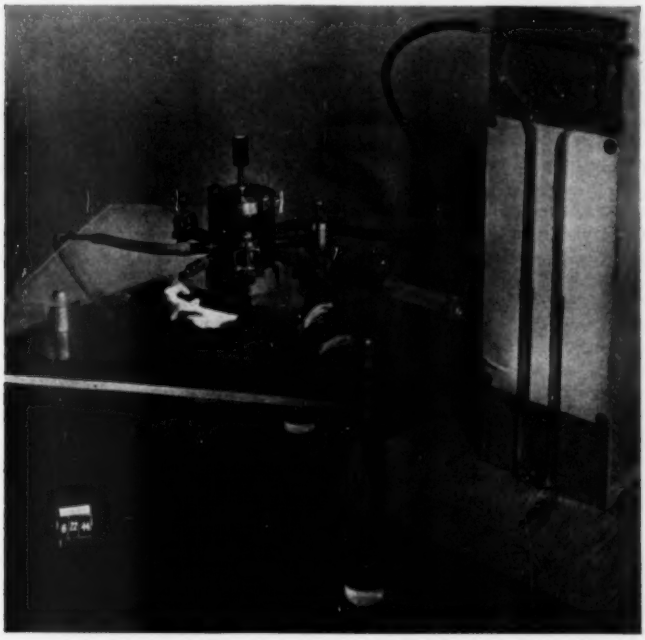

Fig. 4.—Photograph showing the complete aerosol exposure system. The anesthetized rat is breathing oxygen, at a rate illustrated by the flowmeter, along with the active aerosol produced in the electric arc.

in the animals by injecting 80 per cent alcohol (p_H approximately 8) into them through the trachea. Zenker-formaldehyde solution could not be used as a fixative, because it was found to cause fogging of the x-ray film. Formaldehyde solution U. S. P. could not be used, because it leached plutonium from the tissues. After 24 hours' fixation, the lungs were dehydrated in dioxane and embedded in paraffin. Sections 10 microns in thickness were cut and mounted on microscopic slides. The paraffin was removed with xylene. A thin protective covering of celloidin was used on the section. Each slide was placed in contact with a

piece of "no screen" x-ray film and wrapped in black paper to exclude light. Lead weights were used to press the film and the tissue together. The details of the procedure used have been reviewed elsewhere by Axelrod and Hamilton.[6] After the films were exposed and developed, the lung sections were stained with hematoxylin and eosin. Photomicrographs of representative slides and their radioautographs are presented in this report.

RESULTS

The aerosols studied here were, in general, deposited in the same respiratory areas of the rats despite their rather different origin and physical and chemical properties. Immediately after exposure the major proportion of them were found to be distributed from the nasopharyngeal region down through the bronchial tree and into the alveoli of the lungs. The rate at which this general region functioned in the removal of the aerosols was composed of two very different time intervals. The presence of ciliated epithelial cells allowed almost complete removal of the active particles in a matter of hours. This relatively rapid removal of material deposited on the ciliated epithelium agrees with the ciliary removal rates reported by Proetz.[7] In the regions of the lungs, such as the ductus alveolaris and the alveoli, the removal of any large percentage of an aerosol consumed a range of time of the order of many months. The route of removal was primarily via the bronchial tree. Since plutonium and the majority of the fission products given are not absorbed in the gastrointestinal tract to any extent, the material excreted via the bronchial tree could be detected in the fecal fraction of the excreta.[5a, c] However, the products of fission which are comparatively recent contain appreciable amounts of Ba^{140}, which has a half-life of 12.5 days. In addition, all the solutions contained Sr^{89} and Sr^{90}, which have 55 day and 25 year half-lives, respectively. As shown by Hamilton,[5c] these two elements would have been absorbed to some extent through the lungs had they been in solution, since they are absorbed from the intestinal tract when administered orally. The absorbed portion amounted to from 5 to 60 per cent of the total amount given. Of the absorbed portion, 65 per cent was found deposited in bone. It follows, then, that a low bone deposition of these fission products suggests that they were locked in insoluble particles when administered as aerosols. In these studies the insoluble particles could have been carbon, plutonium or uranium in combination with their oxides.

Plutonium Nitrate ($PuO_2[NO_3]_2$) Sprays.—Table 1 shows the fate of plutonium nitrate when administered to rats as a spray, the plutonium being in the plus six state. At 10 minutes after exposure the lungs and the head, the latter including the region above the trachea, contained approximately the same amount of aerosol. It can be seen that the plutonium was removed from the head quickly and was found in the fecal fraction. After one day the lungs continued to lose plutonium, but at a rate much more slowly than the head. The plutonium which was transported across the capillary barrier of the alveoli by whatever means eventually found its way to bone. This transport was not a great factor in the elimination of the pulmonary plutonium, since the highest value obtained was 12 per cent of the total 64 days after administration. The total fraction of plutonium absorbed from the lungs was probably of the order of 20 per cent of the amount retained in the respiratory tract, since 60 to 70 per cent of plutonium absorbed after intramuscular injection is deposited in bone.

6. Axelrod, D. J., and Hamilton, J. G.: The Radioautographic Technique, U. S. Nav. M. Bull., (Supplement) March-April 1948, p. 122.

7. Proetz, A. W.: J. Laryng. & Otol. **49**:557, 1934.

In addition, the absorption of plutonium occurred promptly, since there was little additional plutonium deposited in bone after the fourth day. The aerosols were removed from the head and the lungs by being passed up the bronchial tree, since they were detected primarily in the feces. This includes the fraction that was initially deposited on the ciliated epithelium of the upper respiratory tract and was rapidly swept out, as well as that retained and slowly eliminated from the alveoli.

Radioautographic studies were made at 10 minutes and 16 days after exposure. At the 10 minute interval the ciliated passages of the lungs, as well as the alveoli, contain deposits of plutonium (fig. 5*A*). At 16 days, however, deposition appears to be primarily in areas included in the ductus alveolaris and its primary lobules (fig. 5*B*). The data shown in table 1 demonstrate a reduction of plutonium for all areas of the lung.

Plutonium Oxide Aerosols Prepared from Plutonium Metal and Salts of Plutonium.—Plutonium oxide particles were produced by burning plutonium with graphite in an electric arc in an atmosphere of oxygen. In this case plutonium was presented to the rats in the plus 4 state and probably as some plutonium metal. Its chemistry in the plus 4 state is somewhat similar to the lanthanide series of rare earths. This is in contrast to the plus 6 state of plutonium resulting when the plutonium nitrate sprays were used. The latter compound, $PuO_2(NO_3)_2$, is more

TABLE 1.—*Fate of Plutonium Inhaled as Plutonium Nitrate Spray*

	Percentage of Total per Organ at Given Time After Exposure of Rats				
	10 Min.	1 Day	4 Days	16 Days	64 Days
Lungs	48	39	28	21	9
Head	37	4	1.2	0.6	0.1
Bone	5	8	11	7.9	12
Daily rate of elimination		23	3	0.9	0.3

similar in chemical characteristics to uranyl salts of uranium. The particle sizes produced by the electric arc varied from 0.06 to 38 microns. Their average size was 0.49 micron (fig. 6).

Tables 2, 3 and 4 summarize the results obtained with plutonium oxide aerosols. It can be seen that the results are similar to those obtained with the plutonium nitrate sprays with the exception that relatively little of the aerosol was absorbed into the blood stream, as evidenced by minimal deposits in the bones. Consequently, less was found in the organ of primary deposition, bone. Plutonium oxide aerosols are gradually removed from the nonciliated areas via the bronchial tree. This removal takes a much longer time than that of the aerosols deposited in the portions of the respiratory tract above the trachea, which are included in the head fraction, as well as those deposited on the ciliated epithelium of the bronchial tree. The plutonium oxide aerosols are eliminated in the same manner and at comparable rates whether they are produced by the burning of plutonium salts or by the burning of the metal.

The actual amount of plutonium administered to the rats used in these studies varied from 1 to 3 micrograms per animal. This amount would have given the lungs of the rats about 2 roentgens (r) per day. In this case the electron microscope pictures suggest that some of the aerosol existed as the metal. Furthermore, it is worthy of attention that the aerosol particles appear to follow the same path whether they are detected by following the alpha activity of the plutonium or the beta and gamma radiations of the fission products incorporated in the plutonium

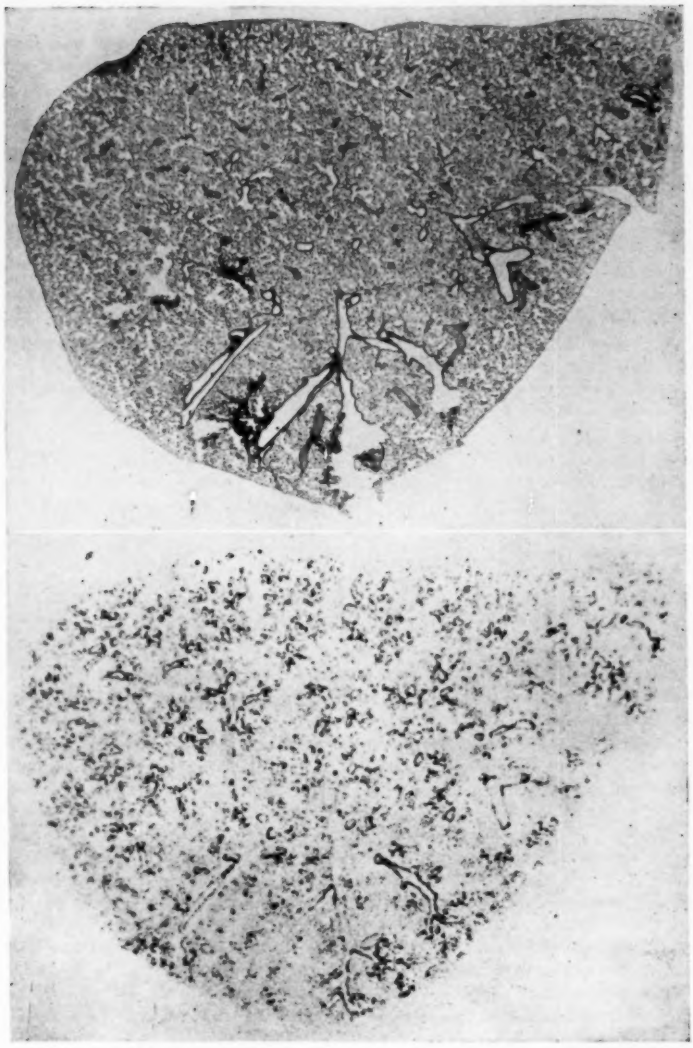

Fig. 5 *A*.—Radioautograph (lower) and lung section (upper) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium chloride and plutonium nitrate. The rat was killed ten minutes after exposure. Plutonium is heavily deposited on ciliated bronchial surfaces and in alveoli. (× 6.)

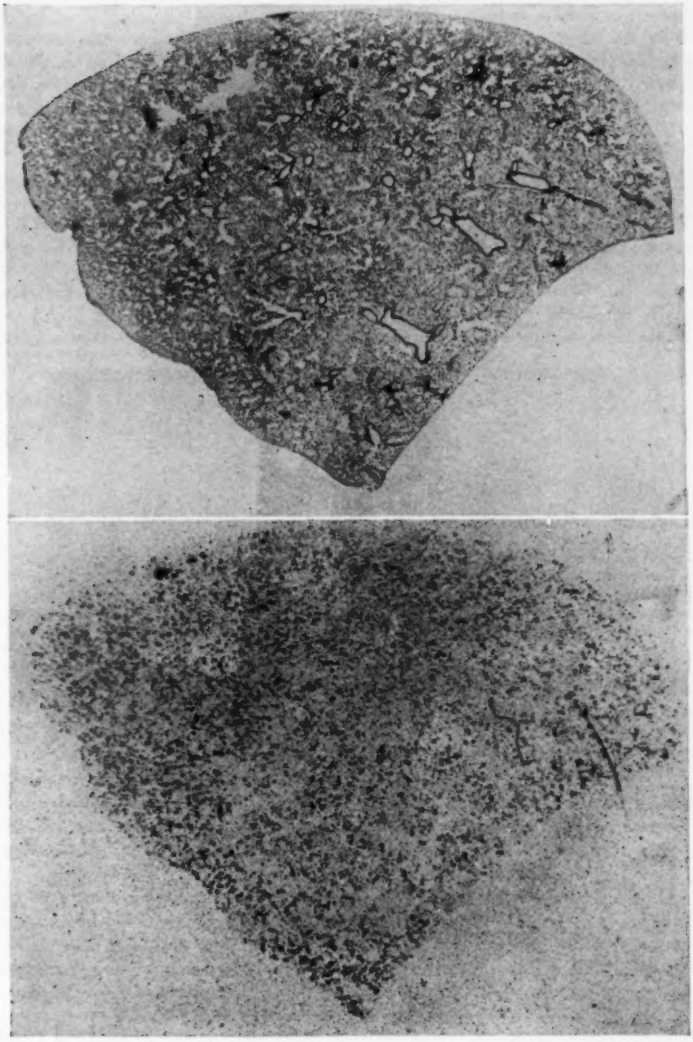

Fig. 5 *B*.—Radiograph (lower) and lung section (upper) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium chloride and plutonium nitrate. The rat was killed sixteen days after exposure. Plutonium has been almost completely cleared off of the ciliated surfaces and is located in alveolar structures. (× 10.)

particles. These results are in agreement with our view that the particles themselves were being followed rather than any one element or group of elements, since the handling of fission products of this age would have been different from that of plutonium (also the carrier-free fission products mentioned on an earlier page [5]). Less than 2 per cent of the total material administered as plutonium was absorbed through the lungs to enter the blood stream and be deposited in the skeleton. With respect to the fission products, the amount thus absorbed was about 7 per cent.

Radioautographic studies suggest that the alveoli were the major area of retention of the inhaled plutonium fission product aerosols after the material laid down on the ciliated passages of the lungs had been expelled (figs. 7 *A*, *B*, *C* and *D*). At the time of exposure 10 fission product disintegrations were occurring for each alpha particle emitted from the plutonium. The plutonium was prepared by placing plutonium metal in graphite electrodes. These were placed in the deuterium oxide pile at the Argonne National Laboratories. At the time of the production of the aerosols the fission products had an average age of 2 months. We estimate

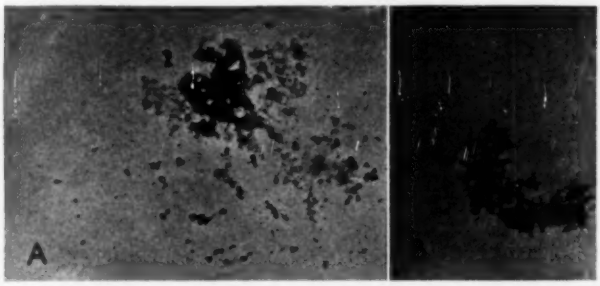

Fig. 6.—Electron micrographs of plutonium oxide aerosols produced by burning plutonium metal on graphite electrodes in an electric arc. (× 8,000.)

that 25 per cent of the film darkening was due to alpha particles and 75 per cent to fission product particles.

Aerosols Composed of Uranium Oxide Containing Fission Products.—Aerosols similar to those composed of the plutonium oxide fission product particles just discussed were produced by burning uranium wire in an electric arc. Smoke particles produced in this manner would be quite comparable to those which might arise from accidental destruction of a uranium pile. The uranium wire had been previously placed in the Clinton pile at Oak Ridge, Tenn., and the fission products were produced by prolonged neutron irradiation. About 60 days elapsed between the time of their removal from the pile and the production of aerosols for inhalation. Although the particle sizes produced on burning ranged from 0.06 to 9.4 microns, the average particle was larger than those produced from plutonium by about a factor of 2, being 0.97 micron in size (fig. 8). This increase in particle size is apparently reflected in the relatively greater entrapment in the respiratory structures of the head. This greater deposition observed in the head as compared with the lungs agrees with the observations of Barclay and co-workers,[8]

8. Barclay, A. E.; Franklin, K., and MacBeth, R. G.: Am. J. Roentgenol. **39**:673, 1938.

who showed that bismuth carbonate dust particles 2 to 12 microns in size were not deposited past the respiratory bronchioles and with those of Policard,[9] who showed that silica and coal particles larger than 10 to 12 microns were not found in the alveoli of miners. Table 5 summarizes the results obtained by exposing

TABLE 2.—*Fate of Plutonium Inhaled as Plutonium Oxide Aerosols Made by Burning Plutonium Metal Embedded in Graphite*

	Percentage of Total Administered per Organ at Given Time After Exposure of Rats				
	10 Min.	1 Day	4 Days	16 Days	64 Days
Lungs	37	..	42	29	11
Head	58.2	..	3	0.3	0.1
Bone *	1	..	0.5	0.4	0.4
Daily rate of elimination		23.7	0.1	0.4	<0.001

* The relatively higher values obtained for bone 10 minutes after exposure are not considered to be significant, since there is a possibility that the animals were contaminated with the aerosol deposited on the nose and face.

TABLE 3.—*Fate of Plutonium Inhaled as a Plutonium Oxide Aerosol Prepared by Burning Plutonium Metal Embedded in Graphite*

	Percentage of Total Administered per Organ at Given Time After Exposure of Rats					
	10 Min.	1 Day	4 Days	16 Days	64 Days	256 Days
Lungs	34	35	27	29	12	4.8
Head	59	12	0.2	0.2	0.5	0.01
Bone *	1	3	1	0.8	1.5	0.2
Daily rate of excretion		28.5	6.4	1	0.2	0.01

* The values obtained for the skeleton could have been high because of outside contamination of the skeleton occurring when the rats were dissected at 10 minutes, 1 day and 4 days after exposure.

TABLE 4.—*Comparative Fate of Plutonium Oxide Aerosols Containing Products Created by Fission of Plutonium Metal in a Deuterium Oxide Pile*

	Percentage of Total Administered per Organ at Given Time After Exposure of Rats					
	10 Min.		1 Day		4 Days	
	As Pu	As F. P.	As Pu	As F. P.	As Pu	As F. P.
Lungs	41	43	..	..	61	56
Head	36	40	..	..	2.4	2.4
Bone *	2	3	..	..	2	7
Daily rate of excretion			19	23	0.5	4
	16 Days		64 Days		128 Days	
	As Pu	As F. P.	As Pu	As F. P.	As Pu	As F. P.
Lungs	16	17	8	9	15	9
Head	0.3	0.3	0.2	0.1	0.1	0.3
Bone *	2	2	0.01	0.3	0.01	0.4
Daily rate of excretion	0.3	0.9	0.2	0.2	0.5	0.1

* The values obtained for the skeleton could have been high because of outside contamination of the skeleton occurring during dissection of the rats at 10 minutes, 1 day and 4 days after exposure.

9. Policard, A.: Bull. d'hist. appliq. à la physiol. **7**:337, 1930; **8**:190, 1930.

rats to uranium aerosols. It can be seen that the elimination of the aerosol is via the bronchial tree and that the rate of elimination is comparable to that of plutonium aerosols. Relatively small amounts of fission products were absorbed and deposited

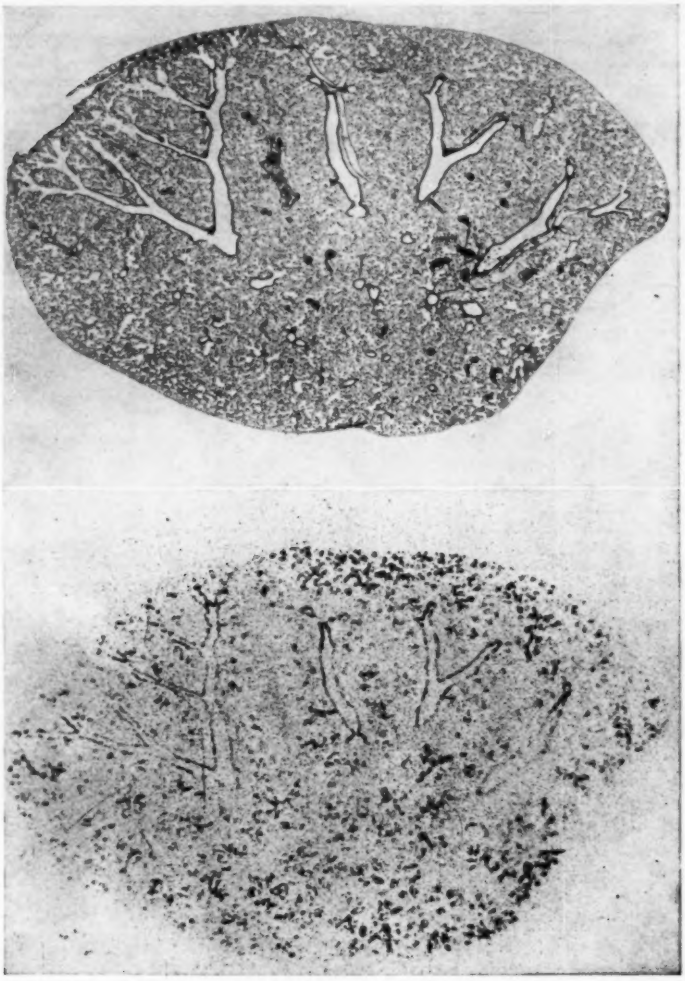

Fig. 7 *A*.—Radioautograph (lower) and lung section (upper) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium metal. The rat was killed ten minutes after exposure. The material is deposited on ciliated surfaces and alveoli. (× 6.8.)

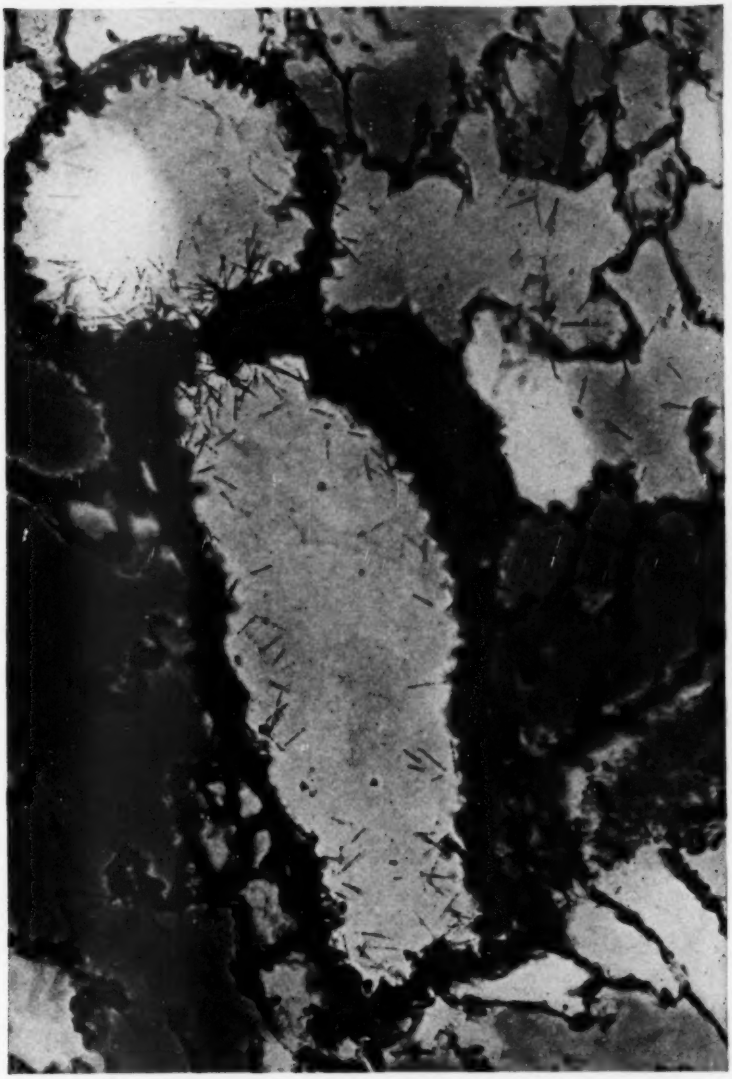

Fig. 7 *B*.—Radioautograph and lung section (superimposed) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium metal. The rat was killed ten minutes after exposure. The tissue section was mounted directly on alpha particle plates. The photomicrograph shows the alpha tracks from plutonium deposited on the walls of two bronchi and on the alveolar epithelium. (× 290.)

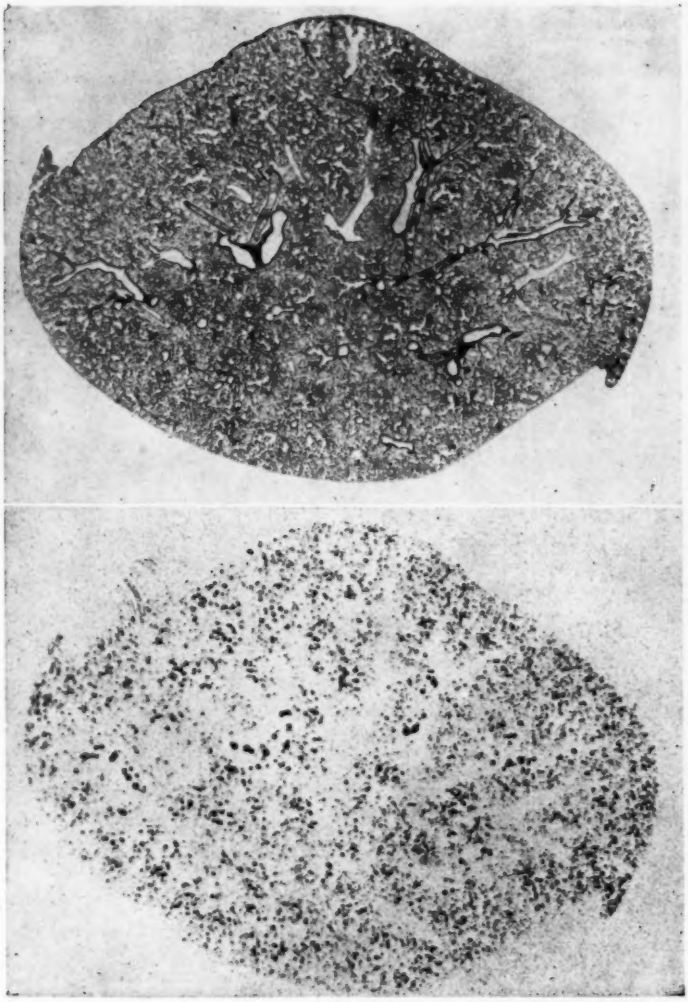

Fig. 7 *C*.—Radioautograph (lower) and lung section (upper) from rat killed one day after inhalation of plutonium oxide aerosol obtained by burning plutonium metal. No plutonium remains on the surfaces of the bronchial tree, but it is arranged in spots throughout all of the alveolar structure. (× 7.5.)

in the skeleton. These results are in contrast with those obtained when solutions of fission products are admitted to the lungs by intubation. About 0.1 mg. of uranium was deposited in the rats along with the fission products inhaled as an aerosol.

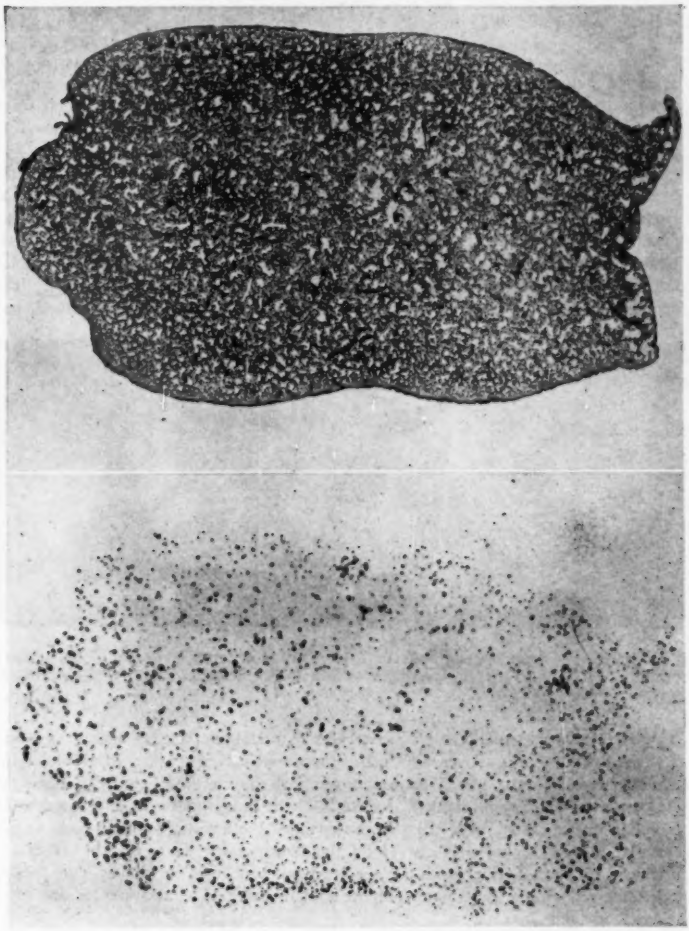

Fig. 7 *D*.—Radioautograph (lower) and lung section (upper) from rat killed sixty-four days after inhalation of plutonium aerosol obtained by burning plutonium metal. A considerable amount of plutonium remains in the lung scattered throughout the alveolar structure. (× 8.)

This amount of uranium produced no apparent toxic effect; the amount of radiation arising from the fission products was below the accepted tolerance level of 0.1 r per

day. In this case the percentage of fission products deposited in bone was much greater 4, 16 and 64 days after administration, as can be seen from an examination of table 5.

This suggests that the uranium oxide particles occluded the fission products and that detection of the fission products was in reality detection of uranium oxide particles.

The radioautographic studies shown in figures 9*A* and 9*B* demonstrate the areas of pulmonary deposition at 10 minutes and at 1 day after exposure to aerosol of uranium oxide containing fission products. The areas of deposition are the same as those previously noted, primarily the alveoli after removal of material deposited on ciliary epithelium.

The aerosols mentioned previously have been composed primarily of the oxides of plutonium, the oxides of uranium, fission products and plutonium nitrate. Additional studies were performed in which the active particles were fission products in the carrier-free state; thus they contained relatively little mass and no plutonium or uranium.[10] Since they were burned with graphite electrodes, the only particulate matter incorporated with the fission products was carbon. The actual number of radioactive atoms which were traced was relatively small, less

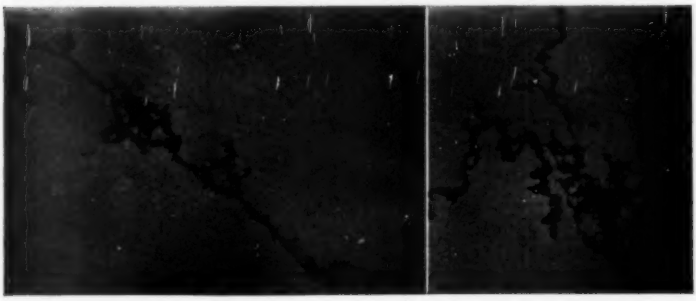

Fig. 8.—Electron micrographs of fission product aerosols prepared by burning uranium metal plus fission products in graphite electrodes in an atmosphere of oxygen. (× 8,000.)

than 10^{13} atoms to each rat. With respect to plutonium, 1 to 3 micrograms was deposited in the lungs of rats during the exposure. About 0.1 mg. of uranium was administered with the fission product uranium oxide aerosols studied. Since the major part of the fission products produce insoluble oxides on burning, they probably remained incorporated with the carbon particles themselves, although this is not certain; they were not disposed of by the lungs in the same manner as fission products admitted to the lungs by the tracheal intubation previously cited. The data obtained on the deposition and the fate of aerosols of this nature are summarized in table 7.

Relatively small amounts of fission products were absorbed into the body and deposited in bone. This statement of course must be qualified with respect to the age of the fission products used. In this case the fission products were 2 to 3 months old when incorporated with plutonium and uranium aerosols. The fission products used in the carrier-free state were from 6 months to 1 year old. For

10. The carrier-free fission products were prepared at the Clinton Engineering Works, Oak Ridge, Tenn.

this reason of age, the most abundant fission products which were present when the radioactive assays were made included strontium, yttrium, zirconium, columbium, ruthenium, cesium, cerium and element 61. Ruthenium and cesium were found to be rapidly eliminated and not retained by the skeleton. The major portion of the material was eliminated via the bronchial tree. The average size of the particles was found to be 0.63 micron and representative pictures are presented in figure 10.

TABLE 5.—*Distribution of Carrier-Free Fission Products Administered as a Chloride Solution by Pulmonary Intubation*

	Percentage Remaining in Whole Organ at Given Number of Days After Administration		
	4 Days	16 Days	64 Days
Lungs	40	21	7
Trachea	3	0.1	0.2
Bone	35	61	74

TABLE 6.—*Fate of Uranium Oxide Aerosol in Rats When the Uranium Oxide Particles Are Followed by the Determination of Radioactivity of the Included Fission Products*

	Percentage of Total Administered per Organ at Given Time After Exposure				
	10 Min.	1 Day	4 Days	16 Days	64 Days
Lungs	12	10	5	7	4
Head	88	7	<0.01	<0.01	0.08
Bone	0.5	1.4	1.9	1.3	1.0
Daily rate of excretion		42	3	5	0.1

TABLE 7.—*Fate of Aerosols Composed of Graphite and Carrier-Free Fission Products When Burned in an Electric Arc in an Atmosphere of Oxygen and Inhaled by Rats*

	Percentage of Total Aerosol Administered per Organ at Given Time After Exposure				
	10 Min.	1 Day	4 Days	16 Days	64 Days
Lungs	65	..	..	25	6
Head	15.0	..	..	< 0.01	<0.01
Bone	1.5	..	..	7.0	7
Daily rate of excretion		30	4	0.8	0.2

In addition to the studies described to this point, a series of rats was exposed to protoactinium. This radioelement, Pa^{233}, in the carrier-free state, was administered to rats as an aerosol by driving it off of gold electrodes in an electric arc in an atmosphere of argon and, of course, eliminating the carbon in the aerosol. Less than 0.001 microgram of protoactinium was administered to these rats, and it was presumably in the form of protoactinium oxide (Pa_2O_5). However, this material could have combined with particulate material and/or bacteria, the presence of which has been demonstrated in the lung by Jones.[11] At any rate, protoactinium, even though in small amount, is not absorbed into the blood stream from the lung

11. Jones, F. S.: J. Exper. Med. **36**:317, 1922.

to any great extent. The results obtained on protoactinium are summarized in table 8.

As with the aerosols discussed earlier, protoactinium aerosols produced in the manner described in the foregoing paragraph are not absorbed into the body from

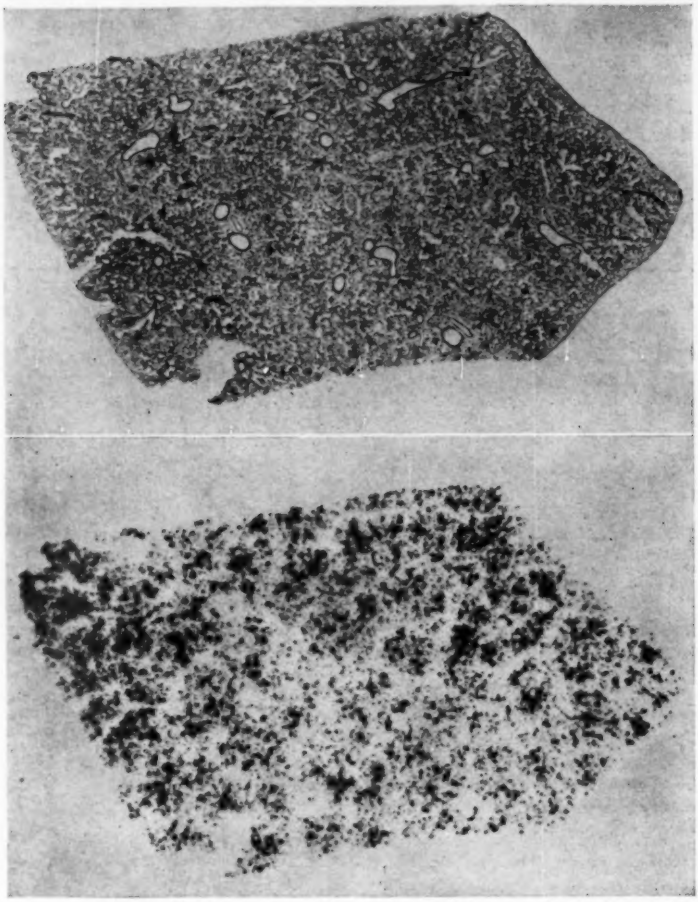

Fig. 9 *A*.—Radioautograph (lower) and tissue section (upper) showing the pulmonary deposition of fission product aerosol obtained by burning uranium metal plus fission products. The rat was killed ten minutes after exposure. The fission product mixture was deposited on ciliated surfaces and throughout the alveolar structure. (× 6.8.)

the lungs but are eliminated rapidly from the head and more slowly from the lungs via the bronchial tree.

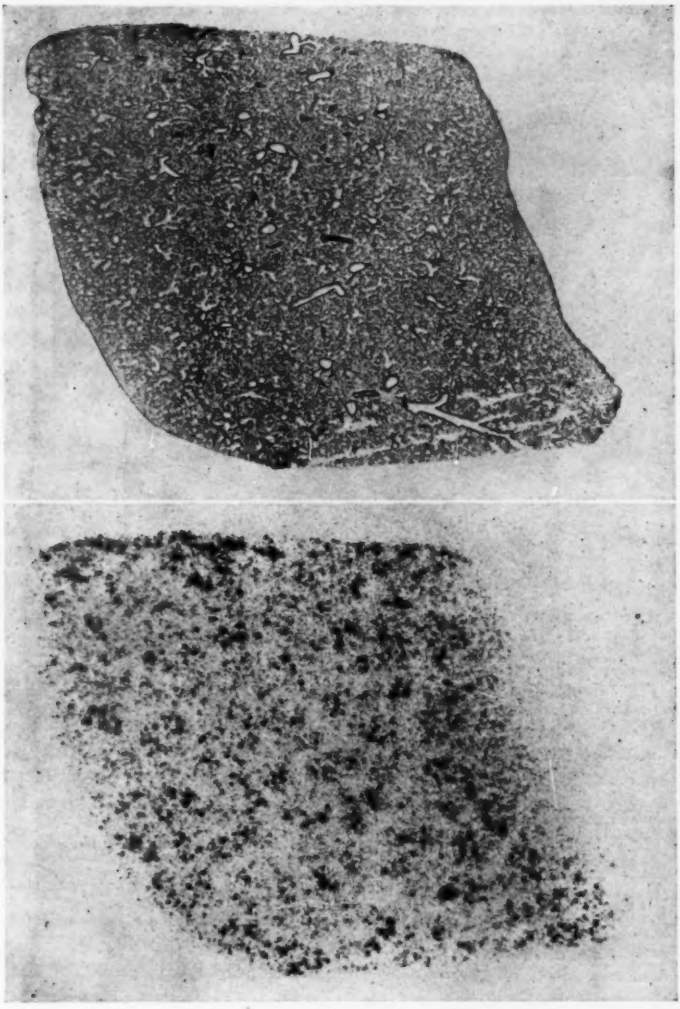

Fig. 9 *B*.—Radioautograph (lower) and tissue section (upper) showing pulmonary deposition one day after exposure to fission products from burning uranium metal plus fission products. The material is almost completely cleared from ciliated surfaces and is scattered throughout the alveolar structure. (× 8.)

It is of interest to know how much of an aerosol is retained during one inspiration and how much is eliminated in the following expiration. It is realized, of course, that the size of particles, the experimental subject used and the velocity of the pulmonary air currents will also modify the results. Some information was obtained from rats exposed under the experimental conditions noted in the section

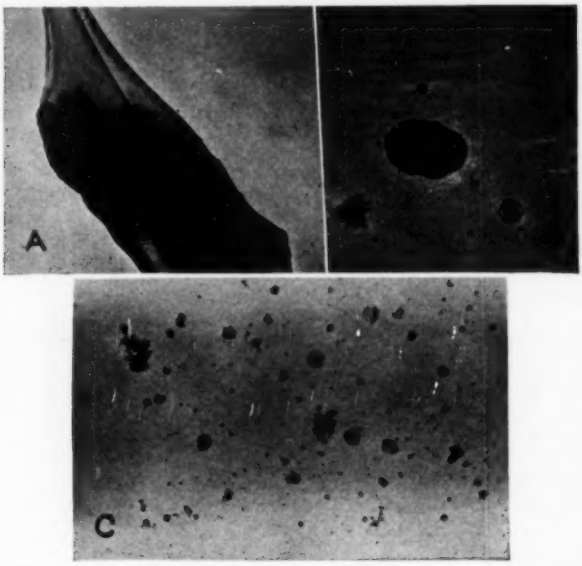

Fig. 10.—Electron micrographs of aerosols of carrier-free fission products evaporated on graphite rods and burned in an electric arc. (× 8,000.)

TABLE 8.—*Fate of Protoactinium Aerosols When Produced by High Voltage Electric Discharge Between Gold Electrodes in an Atmosphere of Argon and Inhaled by Rats*

	Percentage of Total Aerosol Administered per Organ at Given Time After Exposure				
	10 Min.	1 Day	4 Days	16 Days	64 Days
Lungs	42	..	46	39	7
Head	39	..	0.8	0.4	0.4
Bone	0.3	..	3.1	0.9	1.1
Daily rate of excretion		25	2	0.5	0.5

under "Methods of Study" by constructing a valve system which allowed the material exhaled by the rat to be collected separately from that inhaled. When plutonium oxide particles were used, 63.4, 87.6 and 66.5 per cent of the total aerosol inhaled was initially retained in the animal. The particles exhaled were 0.5 to 1.0 micron in diameter, and representative electron micrographs are presented in figure 10.

Similar studies were done on rats and on one of us, carrier-free Zr^{89} with a half-life of 78 hours being used. The Zr^{89} was dispersed as an aerosol, probably as ZrO_2, by the use of the electric discharge in argon described earlier in the protoactinium experiments. Although the material was carrier free, it was not entirely free of foreign particles. These traces of foreign material were introduced while Zr^{89} was being chemically separated from the target material used, which was deuteron-bombarded yttrium oxide. In the single human study done, all but 2.2 per cent of the activity inhaled was retained. Thirty-two per cent was removed via the bronchial tree in 71 hours. This left 66.7 per cent of the activity estimated to have been given remaining in the lungs, presumably in the alveoli.

The parallel experiments in rats showed that 24 per cent of the zirconium aerosol was deposited in the lungs and 40.9 per cent in the head; 28.8 per cent was exhaled and not retained. After 3 days, the lungs contained 18.9 per cent and the head 2.5 per cent, and 60.6 per cent had been eliminated via the bronchial tree, while 3.3 per cent of the Zr^{89} apparently had been absorbed and deposited in the skeleton.

COMMENT

The preceding experiments demonstrate that particulate material 1 micron in size and smaller, composed of plutonium or uranium and/or their fission products, which include a large series of elements in the central region of the periodic table, as well as protoactinium, are deposited in the alveolar structures of the lung, as well as in the air-conditioning passages of the lungs containing ciliated epithelium. This fact is borne out by the radioautographic evidence presented, as well as by the different elimination rates of ciliated epithelium as compared with other structures of the lung, including the ductus alveolaris and beyond. Despite their different chemical and physical natures, the aerosols used in these studies were eliminated from the lung primarily via the bronchial tree at comparable rates. Although the mechanism by which these aerosols were eliminated from the lung was not the problem at hand in these studies, quantitative data were obtained demonstrating their removal and qualitative data indicating their areas of deposition. It would appear to us that the aerosols used, under the experimental conditions of these studies, are removed via the bronchial tree, which is one of the routes suggested by Carleton [12] and others.[13]

No evidence was obtained that any large percentage of the material used in these studies found its way to the lymphatic system of the lungs. In fact, this appears unlikely, since the particles inhaled were continuously removed via the bronchial tree as long as 8 months after exposure. This suggests that the particles remained on the air side of the alveolar structures. The mechanism of their removal appears to be

12. Carleton, M. H.: J. Hyg. **22**:438, 1923-1924.

13. Gardner, L. U.: Am. Rev. Tuberc. **4**:734, 1920-1921. Lemon, S. W., and Higgins, G. M.: ibid. **28**:470, 1933. Drinker, C. K.: J. Indust. Hyg. **3**:295, 1922. Pennar, H. H.: J. M. Research **42**:9, 1920.

the same with respect to the aerosols studied here. This can be demonstrated by plotting all of the pulmonary elimination data on one curve. This is presented in figure 11. Any of the data presented in this report would fall on this curve despite the difference of the materials used in the production of the aerosols.

SUMMARY

The purpose of these experiments was to ascertain the possible hazard resulting from inhalation of fissionable materials and fission products. Aerosols of plutonium, uranium plus fission products, and protoactinium were administered to rats. A Zr^{89} aerosol was administered to a human subject and to rats. Aerosols of the aforementioned elements were almost totally retained by the head and the lungs immediately after exposure. After four days the lungs contained the largest percentage of these elements. The elements deposited in the head and bronchial tree were quickly eliminated via the gastrointestinal tract. The same avenue of elimination was presumably used by the nonciliated portion of the lungs, but at a slower rate. The small percentage absorbed into the body was primarily deposited in the skeleton after conditions of equilibrium had been established. Radioautographic studies indicate that the pulmonary site of deposition of these materials is in the bronchial passages and the alveolar structures. The materials are rapidly removed from the bronchial tree, presumably by ciliary action, and are slowly released from the alveoli. No accumulation of any of the radioelements was observed in either blood vessels or lymph nodes.

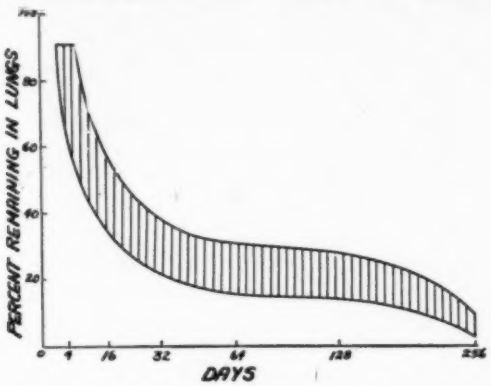

Fig. 11.—Elimination of inhaled radioactive aerosols. The graph represents the data obtained from rats which had inhaled aerosols containing plutonium (in the plus 4 and plus 6 state), uranium, plutonium fission products, uranium fission products and carrier-free fission products.

NEW ELASTIC FIBERS FORMED IN THE WALL OF THE GALLBLADDER IN DISEASE OF THE GALLBLADDER

Pathologic, Clinical and Roentgenologic Data Concerning a Hitherto Neglected Lesion

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IT IS A well known histologic fact that, compared with the respiratory and the anterior digestive tract, the posterior alimentary tract is poor in elastic tissue. The gallbladder, a derivative of the intestine, does not stand as an exception. According to Maximow and Bloom [1] and Hass,[2] there is normally a network of elastic fibers which accompanies the smooth muscle bundles and, also, there are a few elastic fibrils elsewhere, particularly in the perimuscular connective tissue layer. However, in my experience the said network and fibrils are to be seen only under high magnification, at least in the human species. At lower magnification, namely, less than × 60, only the elastica associated with the vessels and the peritoneal lining can be perceived.

The scarcity of elastic fibers may explain why pathologists have apparently failed to investigate its morbid variations. To my knowledge, new formation of elastica in the wall of the gallbladder has been mentioned for the first time only recently, namely, in the second edition of the Chiray, Pavel and Lomon [3] treatise: "La vésicule biliaire et ses voies d'excrétion," issued in 1936. Lately I [4] have published more detailed studies on the same subject. The newer textbooks are entirely silent on the matter.

However, disease of the gallbladder is often attended with definite changes in the amount of elastin. In old hydrocholecystis fibrous degeneration leads finally to diminution and disappearance of elastin.

From the Institute of Pathological Anatomy, University of Montreal.

1. Maximow, A. A., and Bloom, W.: A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1939.

2. Hass, G. M.; Arch., Path. **27**:334, 1939.

3. Chiray, M.; Pavel, L., and Lomon, A., in Chiray, M., and Pavel, I.: La vésicule biliaire et ses voies d'excrétion, ed. 2, Paris, Masson & Cie, 1936, pp. 335-336.

4. Riopelle, J. L.: J. de l'Hôtel-Dieu de Montréal **12**:1, 1942; Union méd. du Canada **75**:1486, 1946.

In other conditions, on the contrary, there is a definite increase in elastica, so that suitable staining methods show deposits of that substance even at low magnification.

It is the purpose of the present paper to bring forward new data on the increase of elastic tissue in the wall of the gallbladder and to discuss anew its genesis and evolution.

MATERIAL AND METHODS

The present paper is based on a study of 1,017 cases of disease of the gallbladder, a number which represents nearly all the cases in which cholecystectomy was done in the Hôtel-Dieu de Montréal, in the years 1944, 1945, 1946 and 1947. The specimens were generally emptied of their contents immediately after their removal and allowed to retract freely. They were afterward incised lengthwise and fixed in Bouin's fluid except in rare instances in which formaldehyde solution or formaldehyde-Müller fluid was used. Stretching or kinking of the viscus was avoided. Length was measured after fixation, while thickness of the wall was computed on histologic sections. Three whole transverse sections were made, in each third of the gallbladder. Tissue blocks were embedded in paraffin. Sections were routinely stained for elastin by Weigert's resorcin-fuchsin technic and counterstained with hemalum, phloxine and saffron. Orcein and Verhoeff's ferric perchloride stain were occasionally used. Out of the general material, series of diverse lengths were taken, for statistical purposes. Histologic selection of cases was done without knowledge of age or sex of patients, or of any other data.

PATHOLOGIC ANATOMY OF NEW GROWTH OF ELASTIC TISSUE

In diseased gallbladders one often finds notable increases of elastica in various locations, namely, in the peritoneal layer of the viscus, in its vessels and, lastly, in the wall proper, outside the vessels.

Thickening of the elastic network of the peritoneum of the gallbladder is frequent in disease of the gallbladder, especially in chronic inflammation. However, this is not peculiar to the serosa of the gallbladder and does not seem to correlate with elastic fiber changes in other layers. For these reasons, I have here only mentioned its existence.

New elastic fibers may also be formed in walls of the blood vessels chiefly in the arteries. Their formation is in no way different from that usually seen in vascular disease, particularly in endarteritis obliterans. This lesion is often most conspicuous in old cholecystitis. There is not any close connection between it and extravascular increase of elastica, as the latter appears much earlier than the former.

Last in line but most important is interstitial production of elastica in the wall of the viscus. The cause, genesis and structure of this increase are peculiar to the gallbladder, so that they deserve special consideration.

There are many modalities of parietal increase of elastic tissue according to distribution and relationship to preexisting structures.

The first variety is seen in more or less localized destruction of the wall, with scar tissue replacement. This is usually the case when calculi are impacted at the neck of the gallbladder, where the mucosa is commonly ulcerated and the rest of the wall is totally or partially replaced by fibrous tissue. In this fibrous tissue one often sees amputation neuromas, hyperplastic endarteritis and considerable

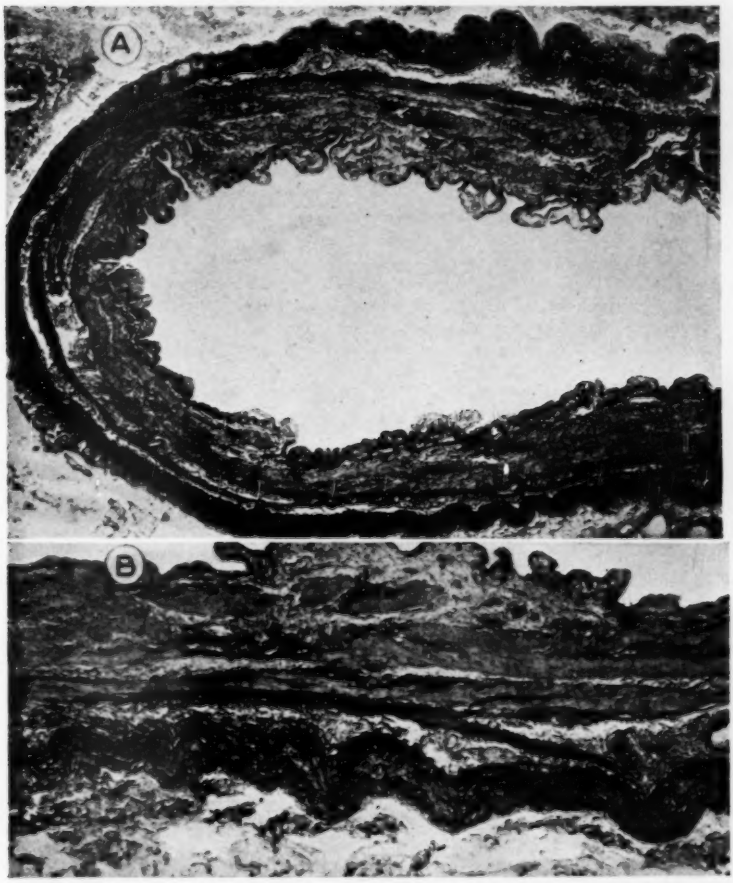

Fig. 1.—*A*, half of a transverse section of the body of the gallbladder; Weigert's resorcin-fuchsin, hemalum, phloxine, saffron; × 25. The mucosal folds are still preserved, although flattened. Numerous elastic deposits are visible in the muscle bundles of the media. A compact wavy band of elastica is seen in the perimuscular tissue layer. In the lower part of the figure the individual elastic fibrils are separated by hyalinized collagen.

B, progressive disappearance of mucosal folds. Moderate hypertrophy of the muscle coat with excess of elastica in a few muscle bundles. Weigert's resorcin-fuchsin, hemalum; × 45. A thin, oblique musculoelastic fascicle joins the media with the perimuscular elastic layer. Aberrant fascicles may be incorporated later to the new-formed elastic layer after disappearance of the muscle fibers. Note the partial hyalinization of the elastic layer.

deposits of elastic substance. In old cases the elastic fibrils curl up in dense, felt-like masses and lose part of their affinity for orcein. The observations that these deposits occur in a destructive lesion, have no organoid structure and show progressive loss of specific tinctorial affinity for orcein point to the degenerative nature of the change, which is reminiscent of elacinic degeneration of the skin.

In most other instances the new elastica shows good affinity for its specific stains, as well as an organoid structure, so that it can hardly be considered as degenerative.

New fibrils may appear more or less diffusely in the common connective tissue of all layers. However, such increase of elastica is slight and can be seen only at high magnification. Its existence or importance is hard to assess in individual cases, owing to the present ignorance of the normal variations according to age, sex and other factors, such as preexisting inflammation.

More important deposits of elastica may be seen at low magnification in specialized connective tissue or in mesenchymal derivatives. They are indeed observed either in the smooth muscle bundles of the tunica media or in the perimuscular connective tissue layer of the outer coat, a layer which is anatomically and functionally associated with the tunica muscularis. Here the deposited elastica, be it diffuse or in multiple separate foci, is not only organoid but also systematized, that is, confined to definite places. As it can easily be seen and graded at low magnification, it is possible to correlate it with various clinical or roentgenologic data.

In the muscle coat the new deposits of elastica assume various forms. The most frequent consists in a thickening and multiplication of the normally minute fibrils coursing along the surface of the smooth muscle cells. Normal or thin muscle fibers persist in the densified elastic network. Usually the change affects a whole muscle bundle along considerable lengths, so that it is seen with elective stains as a black-brown streak running in the middle layer. This change may appear in isolated bundles, or it may affect the greater part of the muscle coat of the gallbladder (fig. 1).

More rarely, the muscle bundles or the whole thickness of the tunica muscularis is locally interrupted through total disappearance of its fibers. Then, its continuity is preserved only by one or many thin streaks of elastica, which is nothing more than the persisting and hypertrophied intramuscular elastic network, emptied of its muscle cells. In other instances the interruption of the continuity of the muscle coat is filled by a scar which is notable because of its high content of elastic fibrils (fig. 2 *B*). Purely collagenous scars are also seen in such locations.

The adventitial increase of elastica appears in the connective tissue sheath which surrounds the exterior surface of the muscle coat and is quite distinct from the loose connective tissue of the subserosa. It consists of elastic bundles made out of parallel wavy fibrils coursing along the external limit of the muscular coat and often connected with it. These fascicles often straddle the large vessels or the branches of the fundamental nerve plexus. In high grade increase the greater part of the adventitia may be occupied by a new-formed elastic layer of remarkable thickness (fig. 1).

Such is the pathologic picture of musculoadventitial elastic hyperplasia. Being organoid, systematized and located in the very places where the elastic network is normally most abundant, the change has more of the nature of tremendous hypertrophy than of that of a degenerative lesion.

Grading of Systematized Musculoadventitial Increase of Elastica.—Nothing is known about the range of variation in the elastic tissue of the normal gallbladder.

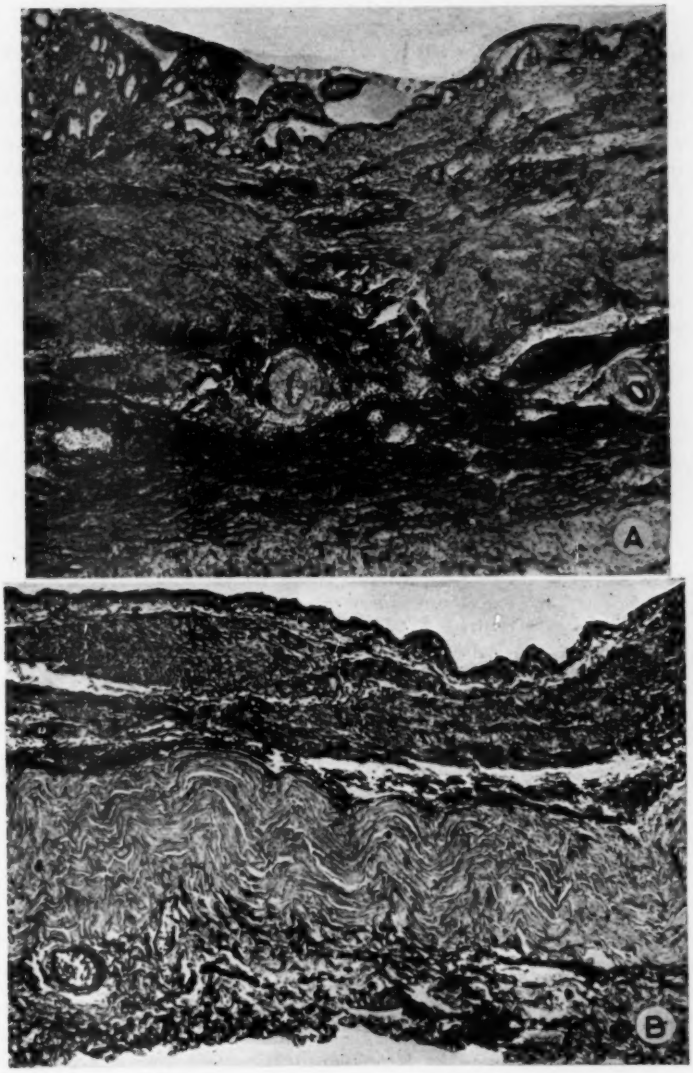

Fig. 2.—*A*, section of the wall of an obstructed gallbladder, with atrophied mucosa but still preserved muscle coat; Weigert's resorcin-fuchsin, hemalum; × 70. A dense, lamellated, wavy band of hyaline collagen runs in the perimuscular tissue layer. Numerous transitional figures show that this is the last stage of hyaline transformation of an adventitial elastic layer.

B, section of the wall of a gallbladder; Weigert's resorcin-fuchsin, hemalum, phloxine, saffron; × 40. In the center, under a localized thinning of the mucosa, the hypertrophied muscle coat is the seat of a narrow vertical scar, made almost entirely of parallel elastic fibrils. A few muscle bundles still bridge the gap in the media. There are a few interfibrillar elastic deposits in the tunica muscularis. In the perimuscular connective tissue layer one sees a thick newformed band of elastic fibers. Thickened arteries and small amputation neuromas are also visible in the same layer.

However, it seems safe to assume that elastic deposits visible at a magnifcation of × 60 are pathologic. At that magnification 55.4 per cent of surgical specimens show no trace of parietal elastica, outside the vessel walls, while 47.6 per cent exhibit various degrees of increase of elastica. One may distinguish minimal elastogenesis (grade 1), which differs from the preceding only because elastica is seen in a few muscle fascicles. In grade 2 many elastic streaks are seen in the tunica muscularis (9.7 per cent), while in grade 3 deposits of elastica are seen also in the perimuscular tissue layer (8.3 per cent). Lastly, cases of grade 4 are those in which, beside more or less developed increase of elastic tissue of the muscular coat, a new-formed adventitial layer is seen, stretching for long distances, in two of three sections (7.3 per cent).

Histogenesis of Systematized Elastic Overgrowth.—According to Chiray, Pavel and Lomon,[8] production of elastic fibrils occurs during subacute cholecystitis, in the edematous young connective tissue which is then present in the adventitia of the gallbladder. There, according to their description, fibroblasts arrange themselves in fascicles, and are progressively transformed into smooth muscle cells, while numerous elastic fibrils appear in their interstices. Thus, the old muscle coat is finally doubled externally by a reticulated musculoelastic layer which may attain considerable thickness. No mention is made of elastogenesis in the pre-existing muscle layer.

In the material of the Institute of Pathologic Anatomy of the University of Montreal, elastic deposits are first observed in the tunica media, as is evident in a study of low grade elastica increase in almost normal gallbladders. Even in cases in which the change is still slight, there is no sign of actual or of past inflammation in the muscle bundles. In most cases there is slight or strong hypertrophy of the tunica media. However, the increase may happen without appreciable hypertrophy. Indeed, morphologic analysis shows that progressive interfibrillar deposition of elastica is accompanied first with thinning, then with atrophy and disappearance of smooth muscle cells.

In the adventitial layer the histogenesis of elastic fibrils is more difficult to elucidate. There is no parallelism between the increase of elastica and the degree of fibrous postinflammatory thickening of the perimuscular layer. Part of the elastic bundles seem to be left over after the disappearance of aberrant muscle fascicles, since many are continuous with oblique muscle tracts connected with the media. Others straddle the big vessels and nerves of the adventitia, far from the media, and so are in locations where muscle bundles are not found. They thus seem to be formed directly from connective tissue, without previous production of smooth muscle.

Fate of Elastic Tissue.—Two possible fates of new-formed elastica may be observed. One is secondary to destructive changes with dense scarring of the wall, and consists in degeneration. It is often seen at the neck of the gallbladder in chronic ulceration due to impaction of a gallstone. The other is progressive disappearance of elastin, which is replaced by fibrohyaline tissue. Such hyaline metamorphosis is seen in the adventitia in some cases of hydrocholecystis and appears as a dense lamellated wavy band of collagen in the adventitia (fig. 2 *A*).

Special Cases and Associated Lesions.—In the series of positive cases on which this article is based, biliary lithiasis was constant, with one exception. In 1 case of papilloma of the gallbladder, increase of elastic tissue, grade 1, was found in a nonhypertrophied muscle coat. In strawberry gallbladders the incidence of new elastic fibers was remarkably low (17 per cent). In high grade increase three wall changes were remarkably frequent, being seen in about half the specimens.

The first was hyperplastic endarteritis; the second, disruption of the muscular coat with development of peculiar transverse elastic scars, as described in a foregoing section; the third was hypertrophy of the nerve plexus in the middle and outer layers, with an occasional amputation neuroma (fig. 3). On the other hand, hyperplasia of the mucosal nerve plexus, a lesion often associated with cholesterosis, was not particularly frequent.

I have observed one surgical and two postmortem specimens of distended gallbladder with stone impaction of the common bile duct and retention jaundice. In those specimens, which were exceptions to Courvoisier's law, there was well developed musculoadventitial overgrowth of elastic tissue. In 1 case of enormous distention of the viscus due to pancreatic carcinoma, there was no increase of elastica, but numerous purely fibrous scars were observed in the muscular coat. One finds increase of elastic fibers in a high percentage of cases of hydrocholecystis. In old cases the wall tends to become entirely fibrous. In intermediate stages one may see progressive disappearance of adventitial elastica

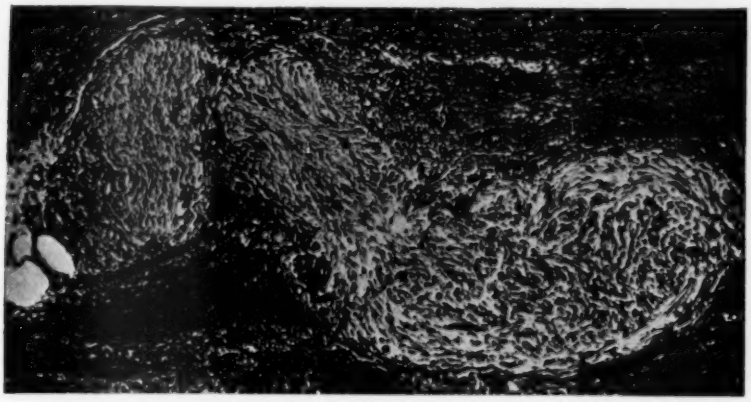

Fig. 3.—Amputation neuroma of the adventitial layer, together with elastic fibers; Weigert's resorcin-fuchsin, hemalum, phloxine, saffron; × 100. Amputation neuromas and hypertrophy of the fundamental nerve plexus are frequent in gallbladders which show high grade increase of elastic fibers and rare in those which show no appreciable overgrowth of elastic fibers.

through hyaline metamorphosis (figs. 1*B* and 2*A*). In 2 cases of mucocele without distention there was no apparent change in the elastic network.

Gross Pathologic Aspects.—There is no characteristic gross picture of fully developed musculoadventitial elastic overgrowth. The mean length of 54 gallbladders in which increase of elastica occurred was 8.2 cm., with variation from 5 to 18 cm.—that is, nearly the same as the mean length of the controls (8.0 cm. with variation from 4 to 12 cm.). The wall is slightly thickened and hardened. In a former work, the mean thickness of the walls of 30 gallbladders in which increase of elastica occurred was 2.6 mm., with extremes of 1.25 and 4 mm., measured on histologic sections. As mentioned in a foregoing section, lithiasis is practically constant. In most cases the bile is more or less diluted. Rarely it is normally colored or colorless.

CLINICAL AND ROENTGENOLOGIC DATA

The sex distribution of grade 4 elastic overgrowth differs from that of the control series, as the ratio of males to females is 1 to 18.25 in the former and 1 to 6.75 in the latter, a discrepancy which is not easily ascribed to random sampling.[5]

The hypothesis that the ratio 1/6.75 is preserved may be rejected at a level of 8 per cent.

In the present series the mean age of patients with grade 4 increase of elastic fibers is 50.67 years at operation, whereas that of persons without increase is 44.68 years, a statistically significant difference.[6] Strawberry gallbladders without increase of elastic fibers seem to be removed a little earlier (mean age of 61 patients, 41.77, which is significant at the 6 per cent level [7]).

The symptoms are not characteristic. However, the general impression is that in the present series of cases as compared with other cases of disease of the gallbladder pain is less dominant, at least in the last stages, the chief complaint being reflex digestive disturbances. More interesting is the study of the mean duration of the clinical history, which is decidedly longer in cases of grade 4. In these cases the mean duration of symptoms is 7.5 years, compared with 5 years in cases without increase of elastica. This difference is statistically significant.[8]

Operation reveals dilatation of the gallbladder in half the cases, and the viscus is described in surgical protocols as big, large, tall, long, dilated, distended, enormous. In only 2 instances was it said to be small, scleroatrophic. In all cases

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5.

	Males	Females	Total Number
f_o	4	69	73
f_{th}	9	64	73

$$\chi^2 = \Sigma \frac{(f_o - f_{th})^2}{f_{th}} = \frac{25}{9} + \frac{25}{64} = 3.2$$

$$\chi^2 = 3.2 \qquad \text{d.f.} = 1$$

6.

Elastic Increase	Patients	Mean Age	σ
Grade 4	73	50.67	11.828
No increase	229	44.69	12.14

$$D = 50.67 - 44.69 = 5.98; \quad \sigma_D = 1.43; \quad \frac{D}{\sigma_D} = \frac{5.98}{1.43} = 4.18.$$

7.

	Patients	Mean Age	σ
No increase of elastica	229	44.69	12.14
Cholesterosis	61	41.77	12.67

$$D = 44.69 - 41.77 = 2.92; \quad \sigma_D = 1.846; \quad \frac{D}{\sigma_D} = \frac{2.920}{1.846} = 1.59.$$

8.

	Patients	Mean Duration of Symptoms	σ
Increase of elastica, grade 4	45	7.5	4.75
No increase	82	5.13	4.74

$$D = 7.5 - 5.13 = 2.37; \quad \sigma_D = 0.716; \quad \frac{D}{\sigma_D} = \frac{2.37}{0.716} = 3.3.$$

NOTE: σ is large relative to the means, indicating that the means may not be very reliable.

save 1 there were calculi in the extrahepatic biliary tract; that is, they were nearly always in the gallbladder; in one fifth of the cases they were in the cystic duct, and in one tenth of the cases, in the common bile duct. Adhesions around the gallbladder are mentioned in one third of the cases.

Roentgenologic examination reveals group differences between cases with and cases without increase of elastica, even if restricted to the study of the degree to which the radiopaque dye is concentrated in the gallbladder. For the sake of comparison, all cholecystograms tabulated here were done at Hôtel-Dieu de Montréal by Dr. Albert Jutras according to his own technic.[9] Dr. Jutras' technic may be briefly described as cholecystography with the patient in upright position and with graduated compression of the gallbladder after two peroral doses of iodoalphionic acid N.N.R. (priodax® [beta-(4-hydroxy-3, 5-diiodophenyl)-alpha-phenylpropionic acid] or dikol® [same chemical composition as priodax®]). The results are shown in table 1.

TABLE 1.—*Roentgenographic Visualization of Gallbladder in Cases with Increase and Cases Without Increase of Elastica*

Cases	Visualization by Cholecystography		
	Normal	Weak	None
No increase in elastica (55 cases)	13 (23.6%)	29 (52.8%)	13 (23.6%)
Increase in elastica, grades 1, 2 and 3 (43 cases)	4 (9.3%)	20 (46.5%)	19 (44.2%)
Increase in elastica, grade 4 (40 cases)	0 (0.0%)	15 (37.5%)	25 (62.5%)

In another series of 56 consecutive cases in which the gallbladder was normally opaque and was removed for calculi soon after cholecystography, the proportion of cases with and cases without increase of elastica was as shown in table 2.

TABLE 2.—*Incidence of Increase of Elastica in Cases in Which Galbladder was Normally Opaque Roentgenographically and Was Removed Because of Calculi*

	Increase		
	No Increase	Grades 1, 2 and 3	Grade 4
56 cases	48 (85.7%)	8 (14.3%)	0 (0.0%)

If there were no relation between increase of elastic tissue and cholecystographic results, the frequency of grades 1, 2 and 3 should be about 40 per cent in the last series, that is, nearly thrice the observed figure. This is further proof of the relative incompatibility of increase of elastic fibers and good roentgenographic visualization.

Filling and emptying time have not been compared up to now in visualized specimens.

PATHOGENESIS OF THE HYPERPLASIA OF ELASTIC TISSUE

The causation of the increase that occurs in the elastica of the diseased gallbladder is still a matter of conjecture, and its complete elucidation would probably require the help of experimentation. How-

9. Jutras, A.: J. de l'Hôtel-Dieu de Montréal **15**:227, 1945.

ever, what is known of the lesion and its etiologic factors justifies a brief discussion of the pathogenesis.

Four different possibilities may be considered in regard to the kind of process which may condition the lesion, namely, degeneration, inflammation, postinflammatory regeneration and reaction to mechanical disturbances.

The first possibility, degeneration, is suggested by the fact that patients with high increase of elastic tissue have a higher mean age at operation, a fact which may point to a certain analogy with senile elastosis of the skin. In the present cases, however, the elastic deposits show an organoid structure which is entirely at variance with the ordinary concept of degeneration and which is completely missing in senile skin. Moreover, the deposits consist of elastin instead of elacin or collastin, as can be shown by differential stains. Typical cases may occur at an early age, and all cases are distributed rather evenly in frequency distribution curves, without undue frequency in old age. In those patients, the higher mean age at operation signifies only that increase of elastica requires some interval of time, and, consequently, that operation has been delayed.

The second possibility is that new formation of elastica is due to inflammation, especially to inflammation of protracted type. That opinion is based on the observation that there is coincidence of increase of elastic tissue and inflammatory changes in the wall of the gallbladder and that there is seeming formation of elastic fibrils in subacute reactions of the adventitial layer. Against that, one may object that the earliest formation of elastica is observed between muscle fibers, without any evidence of inflammation of the tunica muscularis. Coincidence is inevitable because of the frequency with which inflammatory changes occur in diseased gallbladders. These changes or their sequels are neither constantly present with, nor parallel in intensity to, the increase of elastic tissue, as may be seen in fibroplastic pericholecystitis. Lastly, general pathology teaches that, far from being a stimulant to elastogenesis, inflammation per se has destructive effects on elastic networks.

A regenerative, postinflammatory origin is a better founded possibility. It is true that usually scar tissue is almost devoid of elastic fibrils and that regeneration of elastic networks is very late in cicatrices. However, it is a curious property of the fibrous tissue seen in cirrhosis of the liver as well as in scars of kidneys, that it may show conspicuous formation of elastic fibrils (Hass[10]). The connective tissue of the gallbladder may share with that of the liver such a peculiar property.

10. Hass, G. M.: Arch. Path. **27**:583, 1939.

That possibility cannot be ruled out as far as the diffuse formation of elastic fibrils seen in undifferentiated connective tissue of the gallbladder is concerned. However, it seems inadequate in explaining the location and the character of the muscularis and adventitial increase of elastic fibrils and in explaining other, accessory facts, as appears in the last part of the discussion.

The last possibilty, that of a mechanical origin, is based on many concurrent data. These are the tendency toward distention of the gallbladder, the frequency with which calculi are found impacted in the cystic duct, the high incidence of defective roentgenologic visualization in spite of the absence of true cholecystitis, the great variation of content of biliary pigment, all of which point to stagnation of the bile of the gallbladder. The frequent disruptions of the muscle coat and of the deep nerve plexus suggest, also, the possibility of insufficiency and incoordination of muscle contraction. Lastly, the very nature and character of the new-formed substance and its relationship to muscle fibers point to a mechanical determinism in its deposition in the gallbladder wall.

This mechanical condition is probably not a purely continuous stress, that is, simple stretching of the wall, which leads to fibrous atrophy with disappearance of elastin, as is seen in old hydrocholecystis. According to all indications, it requires intermittent dilatation, and ceases after complete anatomic exclusion.

Formal genesis could thus be resumed as a loss of motor control of the gallbladder over its contents and volume, before complete anatomic exclusion of the organ, with intermittent, predominantly passive, volumetric changes, the motor insufficiency being secondary or even primary.

In most cases motor deficiency seems to follow a phase of overwork, which is evidenced by the discovery of a mechanical obstacle, on one side, and by the existence of muscle hypertrophy, on the other. It may then be called secondary motor deficiency. In other cases there is a suggestion of diminution in the power of contraction without sign of previous overwork (primary deficiency). In most instances scars may account for such primary deficiency. In rare cases there is no scarring, no disruption of nerves, no evidence of previous hypertrophy. The muscle is atrophic and partially replaced by elastic tissue. A defective or insufficient stimulation of the muscle coat may then be invoked as a cause of primary insufficiency.

Be that as it may, increase of elastic tissue deserves to take its place among changes which are probably indicative of mechanical perturbance of the function of the gallbladder with stagnation of bile. Its presence may result in preventing excessive dilatation of a permanent and irreversible nature, while the "asystolic" gallbladder is still

connected with the biliary tract. It could then be considered as an adaptive mechanism coming into play when there is a modification between the forces which govern the motions of the gallbladder.

If such is the case, considering the frequency of the lesion and the relative incompatibility of even slight increase of elastica with good roentgenologic visualization of the viscus, one may surmise that mechanical troubles play a larger role, and defective concentration, a lesser one, than formerly thought, in defects of gallbladder visualization.

SUMMARY

In the normal state the wall of the gallbladder is poor in elastic fibers, a fact which may explain the neglect of pathologists in assessing the morbid variations of these components. However, disease of the gallbladder is often attended with slight or pronounced increases of parietal elastica.

The present work describes the pathologic aspects of this neglected lesion, its frequency according to grades, and its histogenesis, as well as the associated alterations of the wall of the gallbladder.

The mean age at operation of patients with high grade increase of elastica is statistically higher than that of patients without such increase. The clinical duration of the disease is likewise significantly longer in the former. There are group differences in the roentgenographic opacity of the specimens between cases without and cases with elastic increase.

The most probable hypothesis concerning the pathogenesis is that there is mechanical trouble in the filling-emptying cycle of the viscus.

PATHOGENESIS OF EXPERIMENTAL HEPATIC FIBROSIS AND CIRRHOSIS IN THE DOG

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IN THE laboratory of the division of physiology of the University of California Medical School, a study has been made of the reaction of the dog's liver to the following procedures: pancreatectomy [1]; the feeding of a high fat, low protein diet [2]; excision of the thyroid and/or the pituitary gland.[3] The collection of these cirrhotic livers during the past has provided a unique opportunity for critically comparing the different forms of cirrhosis that can be produced in the dog and for comparing these reactions with other experimentally induced hepatic lesions described in the literature.[4] Through this comparison it has been possible, for the first time, to distinguish and define clearly the genesis of several forms of fibrosis in the dog and to establish the relation between the initial reactions and the cirrhosis which ultimately supervenes. This comparative study has provided some insight, first, into the significance of hepatocellular damage and circulatory changes in the initiation of hepatic fibrosis and, second, into the relation

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1. Chaikoff, I. L.; Connor, C. L., and Biskind, G. R.: Am. J. Path. **14**:101, 1938.

2. Chaikoff, I. L.; Eichorn, K. B.; Connor, C. L., and Entenman, C.: Am. J. Path. **19**:9, 1943.

3. Chaikoff, I. L.; Gillman, T.; Entenman, C.; Rinehart, J. F., and Reichert, F. L.: J. Exper. Med. **88**:373, 1948.

4. (*a*) Moon, V. H.: Arch. Path. **18**:381, 1934. (*b*) György, P.: Am. J. Clin. Path. **14**:67, 1944. (*c*) Glynn, L. E.: Nutrition Abstr. & Rev. **16**:751, 1946. (*d*) Glynn, L. E.; Himsworth, H. P., and Lindan, O.: Brit. J. Exper. Path. **29**:1, 1948. (*e*) Glynn, L. E., and Himsworth, H. P.: J. Path. & Bact. **56**:297, 1944. (*f*) Zimmerman, H. M., and Hillsman, J. A.: Arch. Path. **9**:1154, 1930. (*g*) Mallory, F. B.; Parker, J., Jr., and Nye, R. N.: J. M. Research **42**:461, 1920-1921. (*h*) Chaikoff, I. L.; Entenman, C.; Gillman, T., and Reichert, F. L.: Proc. Soc. Exper. Biol. & Med. **67**:345, 1948. (*i*) Chaikoff and others, footnotes 1, 2 and 3.

between the etiologic factors and the form of cirrhosis which is detected at autopsy.

NOMENCLATURE

At the outset it is necessary to define the terms to be used here. The term "hepatic fibrosis" will be applied to a histologically detectable increase of the connective tissue of the liver. When all states have been observed in the same liver between the earliest detectable increase of connective tissue and frank cirrhosis, the lesion will be referred to as precirrhosis. "Cirrhosis" will be used to describe a marked increase of hepatic connective tissue associated with obvious distortion of the lobular structure, particularly a derangement of the usual relationship between neighboring lobules, or fibrous tissue disfigurement of large numbers of individual hepatic lobules.

Terms like "interstitial fibrosis," "interlobular fibrosis" and "intralobular fibrosis" are so widely used and correctly interpreted in pathology as to require no special mention here.

TYPES AND GENESIS OF HEPATIC FIBROSIS AND CIRRHOSIS ENCOUNTERED IN THE DOG

The forms of fibrosis seen in the dog may be classified into three main groups:

1. Centrolobular or peri–hepatic vein fibrosis.
2. Periportal fibrosis.
3. Diffuse interstitial or intralobular fibrosis.

As will appear from the following descriptions, these names refer to the location in which the excess fibrous tissue proliferation is initiated, for, with the progress of a lesion, one form may become widespread and develop into, or become associated with, a second or a third form. For example, a centrolobular fibrosis may, by extending into the tissue more widely removed from the radicles of the hepatic vein, develop into diffuse interstitial fibrosis. On the other hand, a lesion apparently commencing interstitially some distance from the central vein may later spread to the central region and become, for a time, localized there.

In the very late stages of cirrhosis, when structural distortion is gross, it may be impossible to determine in any single section the mode of development of a particular cirrhotic lesion. However, we have repeatedly found, even in the same section or in sections from different lobes of the same liver, several stages in the evolution of a particular form of cirrhosis.

1. *Centrolobular or Peri-Hepatic Vein Fibrosis* (figs. 1 to 10).—Before 1946 this form of hepatic fibrosis had been described only in

patients with chronic cardiac decompensation [5] and in dogs with surgically impaired hepatic venous return.[4f] However, the recent observations of Lillie and associates [6] were subsequently extended; Ashburn, Endicott, Daft and Lillie,[7] through a series of careful experiments, demonstrated conclusively that in the rat several forms of experimentally induced hepatic fibrosis previously regarded as periportal in origin actually developed around the radicles of the hepatic veins.

In our series of dogs, centrolobular or peri–hepatic vein fibrosis occurred in animals subjected to a variety of experimental procedures, including pancreatectomy, the administration of large quantities of alcohol to animals maintained on a high fat or a high protein diet, and ablation of both the thyroid and the pituitary gland or of the thyroid gland alone.

In the majority of dogs this form of fibrosis was encountered in livers showing various degrees of fatty change. The fatty change was usually most marked toward the centers of the lobules (fig. 1), and it was around the enlarged, very fatty liver cells that the initial changes were usually detected. These early reactions consisted of edema of the small rim of connective tissue and reticulum supporting the central vein, together with a collection of cells, including some polymorphonuclear leukocytes and one or more giant cells (fig. 2). Such cellular infiltration was not regularly encountered in all the affected lobules. In some livers the earliest change was edema and increased eosinophilia or basophilia of the reticular fibers between and around the enlarged, fatty liver cells near the central zone (figs. 3, 4 and 5). This change in the pericellular and intercellular reticulum, detected in routine hematoxylin and eosin preparations, was shown (by special silver methods for demonstrating the reticular fibers) to represent a distinct thickening of the individual argyrophilic fibers, which soon became more numerous, although they occupied the usual intercellular positions. Such thickening and increase of the amount of reticular fibers have been described in human cardiac cirrhosis by several investigators,[5] in dogs [4f] and in nutritional cirrhosis of rats.[6] In our dogs this change of the reticulum was soon succeeded by an actual increase of fibrocytic and fibroblastic cells as well as of monocytes, lymphocytes and thrombocytes, which still occupied intercellular and pericellular positions along the line of the altered reticulum.

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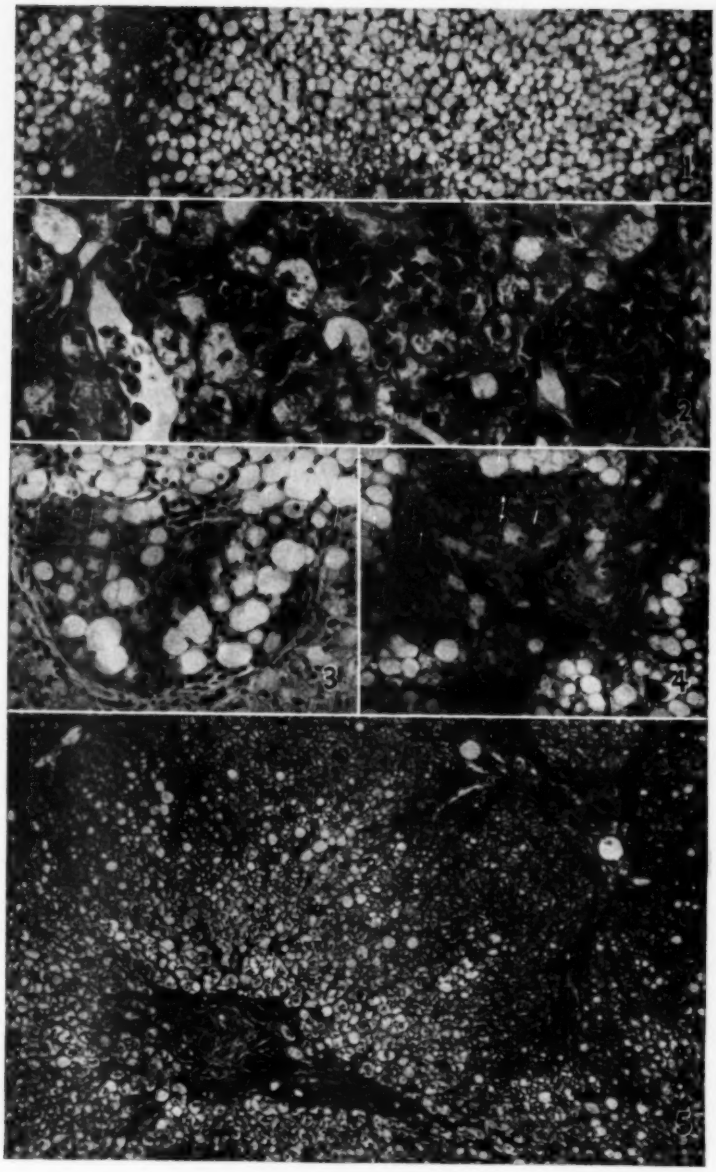
5. Lambert, R. A., and Allison, B. R.: Bull. Johns Hopkins Hosp. **27**:350, 1916. Katzin, H. M.; Waller, J. V., and Blumgart, H. L.: Arch. Int. Med. **64**:457, 1939. Koletsky, S., and Barnebee, J. H.: Am. J. M. Sc. **207**:421, 1944. Costero, I., and Barroso-Miguel, R.: Arch. Inst. cardiol. Mexico **17**:337, 1947.

6. Lillie, R. D.; Ashburn, L. L.; Sebrell, W. H.; Daft, F. S., and Lowry, J. V.: Pub. Health Rep. **57**:502, 1942.

7. Ashburn, L. L.; Endicott, K. M.; Daft, F. S., and Lillie, R. D.: Am. J. Path. **23**:159, 1947.



Figures 1-5

(See legends on opposite page)

This reaction may remain localized and progress extremely slowly, in which case an intralobular fibroma-like lesion may occur. In such instances the enlarged, fatty liver cells remain intact and apparently healthy, although each cell is surrounded by young fibrous tissue (fig. 3). Even the focus of this reaction may be totally encapsulated by a thin rim of fibrous tissue (fig. 3). Within such a fibroid-like lesion, giant cells with single multilobular and contorted nuclei are occasionally encountered. Such lesions, occurring in fatty tissue, have been previously described, so far as we know, only in the subcutaneous fat of the extremities.[8] In the latter site this reaction is succeeded by fibrosis and atrophy of the subcutaneous fat, with consequent puckering of the overlying skin.

In the liver such localization of the initial fibrous tissue reaction is unusual, for as a rule the fibrosis is more widespread and extends fairly rapidly into the adjacent centrolobular fatty liver tissue. In figure 5 the lesion has apparently commenced slowly and remained localized at the outset, as in figure 3, but the reaction has then spread quite extensively among the adjacent fatty parenchymal cells. Figure 4 depicts an intermediary rate of development, in which the fatty cells

8. Gilchrist, T. C., and Ketron, L. W.: Bull. Johns Hopkins Hosp. **27**:291, 1916.

EXPLANATION OF FIGS. 1-5

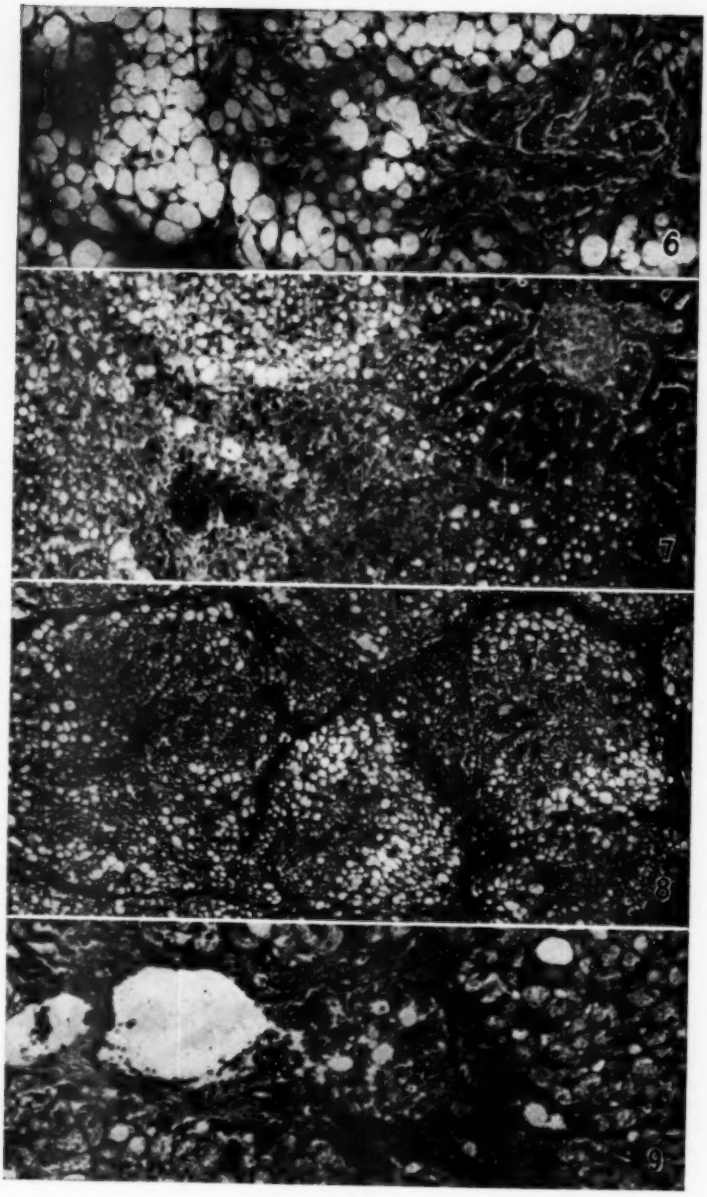
Fig. 1 (dog D28, depancreatized 15 weeks prior to microscopic examination of liver; maintained with insulin and a lean meat diet).—The fatty change is most marked toward the center of the lobule, while the rim of cells toward the portal tracts is virtually free of fat. The central vein is at bottom center. Hematoxylin and eosin; × 115.

Fig. 2 (dog DE, depancreatized 2.3 years prior to examination; maintained with insulin and a lean meat diet).—Focal accumulations of cells, among which are a few polymorphonuclear leukocytes and occasional mononuclear giant cells, near the longitudinally sectioned central veins on the left, such as depicted here, are typical of the early reactions which may be followed by fibrosis. Hematoxylin and eosin; × 340.

Fig. 3 (dog D66, depancreatized 36 weeks prior to examination; maintained with insulin and a lean meat diet for first 20 weeks; raw pancreas added to diet for last 16 weeks).—The fibrosis around the centrolobular fatty liver cells is fairly well advanced, although localized and encapsulated by fibrous tissue. Hematoxylin and eosin; × 140.

Fig. 4 (dog F10, high fat diet plus alcohol for 4 months).—Several stages in the course of centrolobular fibrosis are shown. The central mass of fibrous tissue, with new capillaries, represents a fairly advanced stage of a comparatively localized fibrosis. Around this central mass, and especially toward its lower right corner, can be seen the earlier stages of intercellular edema and thickening of the reticulum, with increased cellularity between the fatty liver cells. Hematoxylin and eosin; × 125.

Fig. 5 (dog HT8, hypophysectomized and thyroidectomized 419 days prior to examination).—Toward the bottom is shown a localized fibroid-like change among the fatty liver cells, with the fibrosis extending in all directions among the fatty cells. An early associated periportal fibrosis is also visible (upper right corner). Hematoxylin and eosin; × 70.



Figures 6-9

(See legends on opposite page)

enclosed within the initially affected area have become completely obliterated by the fibrotic process (compare fig. 3 with intact fatty liver cells). In the latter instance, capillaries have appeared within the focus of fibrosis. Moreover, the earlier stages of the fibrotic process have also made their appearance around the fatty cells immediately abutting on the almost rectangular central area of fibrosis.

When centrolobular fibrosis is acute and highly active, it adopts the form depicted in figure 6. In these instances the alterations of the reticular fibers described in a foregoing paragraph spread rapidly and erratically in all directions throughout the lobule and may soon involve the portal tracts. When the centrolobular fibrosis spreads thus rapidly, it may become difficult to distinguish its site of origin, and consequently such a lesion may be taken to be interstitial fibrosis. However, the character of the new connective tissue in the centrolobular lesion is quite different from that encountered in the forms of interstitial fibrosis described in a later section of this paper (compare figs. 5, 6 and 8 with figs. 16, 17 and 18).

On several occasions we have seen in depancreatized dogs and in animals fed a high fat diet with or without alcohol an acute hemorrhagic necrosis supervening in the centrolobular fatty parenchyma. Such a hemorrhagic change may be widespread throughout the liver or may be confined to one or more lobes. Should the animal survive, the hemorrhage is followed by fibrosis within the centrolobular necrosis resulting from the hemorrhage (fig. 7). In such instances the reaction

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EXPLANATION OF FIGS. 6-9

Fig. 6 (dog D24, depancreatized 39 weeks prior to microscopic examination of liver; maintained with insulin and a lean meat diet for first 33.5 weeks; raw pancreas added to diet for last 5.5 weeks).—The fibrosis around the fatty liver cells is highly active and progressing rapidly. There is an associated early periportal fibrosis. Hematoxylin and eosin; × 130.

Fig. 7 (dog F57, fed a high protein diet plus alcohol for 9 weeks).—In this specimen a centrolobular hemorrhage has occurred, with incipient fibrosis, among the centrolobular fatty liver cells. Note that the periportal areas show no abnormal reaction. Hematoxylin and eosin; × 92.

Fig. 8 (dog HT8, hypophysectomized and thyroidectomized approximately 36 weeks prior to examination).—Fibrosis localized within the area of fatty change is clearly shown. The fibrous tissue in the centrolobular regions of adjacent lobules has now joined to form a fibrous ring enclosing portions of several lobules around centrally located, small portal tracts. Several such fibrous rings portrayed in this picture give the appearance of a monolobular cirrhosis. There is some extension of the fibrosis among the less fatty neighboring liver cells, together with some reaction in one of the portal tracts lying centrally within a fibrous ring. Hematoxylin and eosin; × 70.

Fig. 9 (dog D12, depancreatized 34.5 weeks prior to examination; maintained with insulin and a lean meat diet for 28.5 weeks; raw pancreas added to diet for last 6 weeks).—A branch of a hepatic vein with eosinophilia of the connective tissue and associated fibrosis spreading among the surrounding degenerating liver cells. This peculiar fibrotic change is not obviously related to the fat in the liver cells. Hematoxylin and eosin; × 170.

resembles closely the more acute changes observed by Zimmerman and Hillsman [42] in dogs with surgically induced obstruction of the inferior vena cava. Although in these instances the fibrosis is precipitated acutely after the hemorrhage, the lesion subsequently progresses in a manner almost identical with that described in foregoing paragraphs as occurring in the fatty liver.

Figure 8 shows what happens when the liver is involved in a centrolobular fatty change and fibrosis. Here it can be seen that in each lobule the centrally lying fat has become affected by a fibrotic process which has spread along the radicles of the hepatic veins. Frequently the central vein is not detectable, because of the intensity of the fatty change, and it becomes clear that the larger rami of the hepatic veins are similarly affected by perivascular fat and fibrous tissue changes. Moreover, the fibrosis around the hepatic radicles causes adjacent lobules to join one another, so that rings of fibrous tissue formed through this union of the central zones of three or more adjacent lobules can easily be recognized, the center of the fibrous ring or circle being represented by a portal tract. Superficial examination gives the impression that this is a monolobular cirrhosis, but in actual fact several neighboring lobules are participating in the process (fig. 8).

When the aforementioned lesions progress gradually, their centrolobular or peri-hepatic vein origin can be distinguished without difficulty. However, in the subsequent stages the fibrous tissue rings contract, the fibrosis extends and the portal tracts are drawn into the zone of fibrosis. The centrolobular lesions may often be complicated with the supervention of both interstitial and periportal fibrosis. In such instances the distortion of hepatic structure is not only more impressive but more precipitous, frequently making the distinction between centrolobular and periportal cirrhosis difficult, and can be made out with certainty only by studying the genesis of the lesion in different parts of the same section.

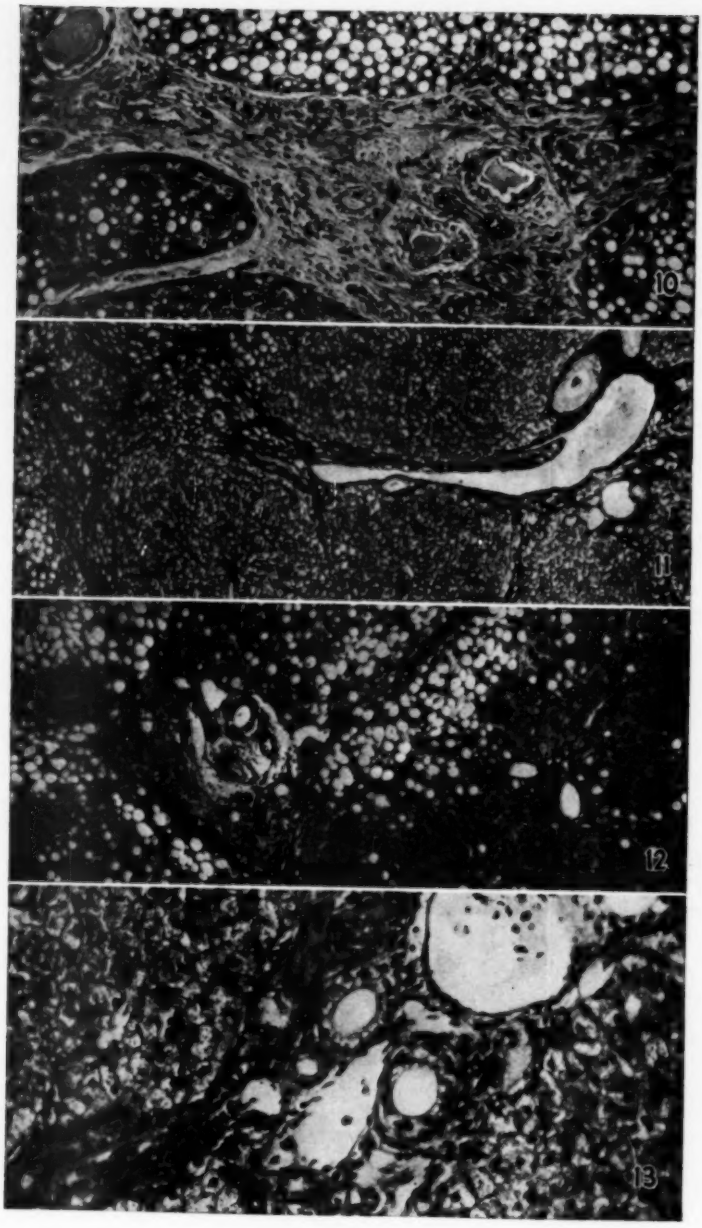
One other fibrotic reaction around the radicles of the hepatic vein has been encountered in dog livers. In this reaction a peculiar form of fibrosis is initiated in the connective tissue which supports the hepatic vein itself. The hepatic cells in this region appear to undergo eosinophilic degeneration, which makes them denser and more obvious. This degeneration is succeeded by an increase of fibrocytes, and later by a highly eosinophilic form of dense connective tissue which can be seen insinuating itself into the surrounding parenchyma (fig. 9). While this lesion usually occurs in a mildly fatty liver, the connective tissue reaction cannot be related in any obvious way to the fatty change. The progress of this form of peri-hepatic vein fibrosis culminates in cirrhosis in the manner described in the foregoing paragraph.

2. *Periportal Fibrosis and Cirrhosis* (figs. 10 to 15).—In its most widely recognized form this fibrotic change in the liver commences with an accumulation of polymorphonuclear leukocytes and other cells in the portal tracts together with edema of the periportal collagen fibers. As has been demonstrated previously, in hypophysectomized and thyroidectomized dogs,[8] periportal fibrosis may, in its initial stages, closely resemble the early stages of the cholangiolitic type of cirrhosis observed in human subjects by Watson and Hoffbauer [9] and regarded by them as secondary to chronic or repeated attacks of hepatitis. It is unlikely, however, that an infective hepatitis could have been the cause in our dogs. Following the cellular infiltration there is an increase in the amount of collagen in and around the smaller portal tracts, and when this connective tissue spreads into the abutting parenchyma and distorts the lobular pattern, cirrhosis supervenes. A similar process has also been seen in depancreatized dogs. In these animals the fibrosis usually occurs in a liver showing predominantly periportal fatty changes (fig. 10). However, in the later stages the relationship between the periportal location of the fat and the advanced cirrhosis is not detectable, since the fatty change affects the liver more diffusely (fig. 11). Thus, while the liver depicted in figure 10 contained 14 per cent total fatty acids, that in figure 11 contained 29 per cent total fatty acids.

In the later stages, too, hyperplasia of bile ducts is commonly seen in the densely fibrotic portal zones (fig. 11). Unless fibrosis commences elsewhere in the parenchyma, i. e., interstitially or centrolobularly, the disease progresses slowly, and structural distortion is long delayed. Such a slowly progressive portal fibrosis may result in a monolobular-like cirrhosis (fig. 12) closely resembling that following slow centrolobular fibrosis (compare fig. 12 with fig. 8). Whereas in figure 12 the central veins are obviously unaffected by the fibrosis and lie in the usual position, in figure 8 the central veins of adjacent lobules have become joined by fibrous tissue which, forming a ring, surrounds the nonfibrotic, centrally located portal tracts. Superficially these two processes resemble one another closely, but careful study reveals their fundamental differences. However, in depancreatized dogs and in normal animals fed a high fat diet the type of interstitial fibrosis described in the following section commonly occurs in association with the periportal changes, and consequently gross structural distortion rapidly follows the combined effects of periportal and interstitial fibrosis.

In depancreatized dogs, still another form of periportal fibrosis may occur in a relatively nonfatty liver. Thus, in figures 13 and 14, which reproduce sections from a liver containing only 10 per cent total

9. Watson, C. J., and Hoffbauer, F. W.: Ann. Int. Med. **25**:195, 1946.



Figures 10-13

(See legend on opposite page)

fatty acids, a striking collection of cells, including polymorphonuclear leukocytes and fibroblasts, can be seen surrounding the portal tracts. This type of periportal fibrosis appears to progress rapidly, resulting in an early uniting, by young fibrous tissue, of the neighboring portal areas (fig. 14). Interstitial and centrolobular fibrosis usually supervenes early and results in rapid distortion or cirrhosis of these nonfatty livers.

The periportal fibrosis may also begin in the manner described for the peculiar pericentral fibrosis depicted in figure 9; that is, the connective tissue around the portal tracts and adjacent parenchyma may undergo thickening and become intensely eosinophilic (fig. 15). With the radial spread of these peculiar changes, fibrosis becomes more extensive and culminates ultimately in cirrhosis. Whereas in figure 9 the reaction is essentially around the hepatic radicles, in figure 15 it is obviously the portal tract which is clearly pathologic.

With periportal fibrosis, as with centrolobular fibrosis, the rate at which gross structural distortion supervenes is determined not so much by the site of inception of the fibrosis as by the extent of the reaction, its speed of progress and the occurrence of fibrosis in other sites. The presence or the absence of lobular hyperplasia will determine to a significant extent the degree of structural disortion and therefore the terminal pathologic picture.

3. *Diffuse Interstitial or Intralobular Fibrosis* (figs. 16 to 21).—This type of fibrosis was most frequent in depancreatized dogs and in normal animals restricted to high fat, low protein diets with or without

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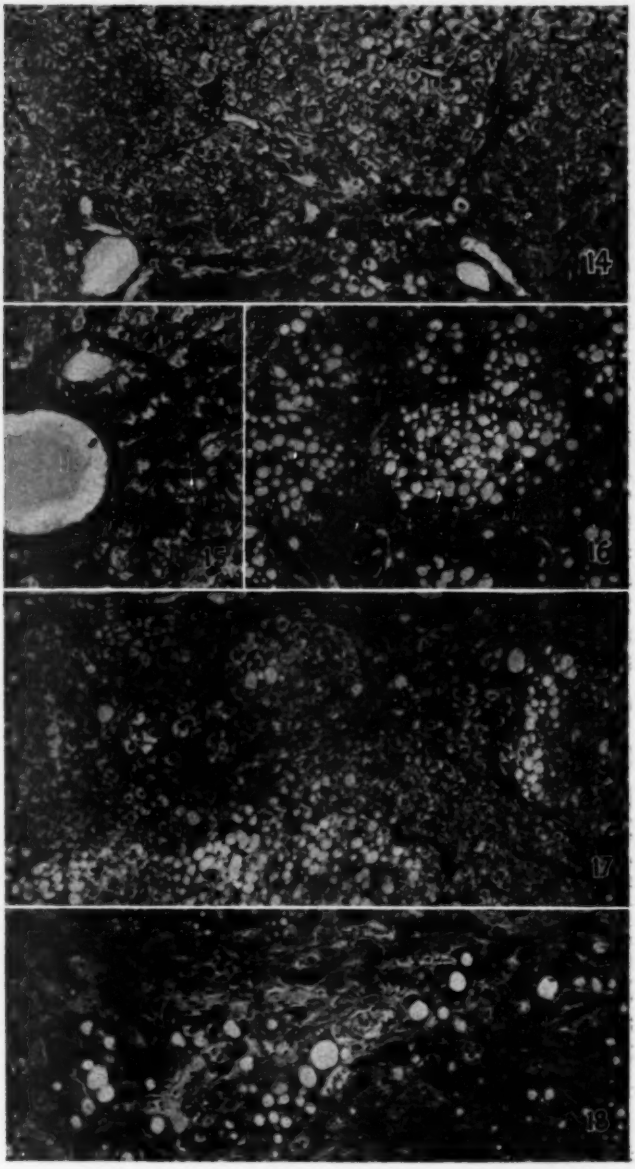
EXPLANATION OF FIGS. 10-13

Fig. 10 (dog D65, depancreatized 35 weeks prior to microscopic examination of liver; maintained with insulin and a lean meat diet for 20 weeks; autoclaved pancreas added to diet for last 15 weeks).—In this liver the fatty change is most marked among the periportal liver cells. The portal tract is edematous and undergoing early fibrosis, with the fibrous tissue extending along the course of the portal triads immediately related to the most severely affected tract in this picture. There is some associated hyperplasia of bile ducts. Hematoxylin and eosin; × 114.

Fig. 11 (dog DG, depancreatized 1.5 years prior to examination; maintained with insulin and a lean meat diet).—Later stage in portal fibrosis with early structural distortion. Hyperplasia of bile ducts has occurred within the densely fibrosed portal areas. Hematoxylin and eosin; × 70.

Fig. 12 (dog DA, depancreatized 5.5 years prior to examination; maintained with insulin and a lean meat diet).—Late stage in portal fibrosis with bands of fibrous tissue accumulating along the portal tracts and surrounding lobules. The central veins in the affected lobules do not show any significant change. Mallory's phosphotungstic acid–hematoxylin; × 70.

Fig. 13 (dog DE, depancreatized 2.6 years prior to examination; maintained with insulin and a lean meat diet for 2.3 years; raw pancreas added to diet for last 16 weeks).—A large number of polymorphonuclear leukocytes and early but distinct fibrosis are detectable. This reaction is not obviously related to the comparatively mild fatty change in this liver, nor can biliary obstruction or infection be regarded as the precipitating cause of this lesion. Hematoxylin and eosin; × 225.



Figures 14-18

(See legends on opposite page)

alcohol.[10] Although in earlier reports [10a, c] on the animals subjected to the treatments mentioned "intralobular fibrosis" was referred to, neither its nature nor its mode of development was fully described.

The livers affected by such diffuse interstitial fibrosis are almost invariably very fatty. In this lesion, numerous small fibroblasts and fibrocytes develop among the enlarged fatty liver cells. There does not seem to be any predilection for a particular site in the liver lobule, for, as can be seen from figures 16, 17 and 18, this peculiar fibrosis may commence anywhere in the lobule. The portal tracts and central veins are involved rather late.

The fibrous tissue may be highly cellular and may spread rapidly through large areas of parenchyma (figs. 16 and 17). In such instances the sections of the liver are so cellular as to resemble diffuse hepatic "sarcomatosis" (figs. 16 to 20). Entire areas of hepatic epithelium are replaced by whorls of highly cellular connective tissue (figs. 19 and 20). In the later stages all that remain of the liver epithelium and bile ducts are scattered small groups of degenerate liver cells and areas of bile duct proliferation (fig. 20).

As can be seen from figures 16 to 20, this diffuse fibrosis is not preceded by, or associated with, focal or massive necrosis such as is

10. (*a*) Conner, C. L., and Chaikoff, I. L.: Proc. Soc. Exper. Biol. & Med. **39**:356, 1939. (*b*) Chaikoff, I. L.; Entenman, C.; Gillman, T., and Connor, C. L.: Arch. Path. **45**:435, 1948. (*c*) Chaikoff and others.[2]

EXPLANATION OF FIGS. 14-18

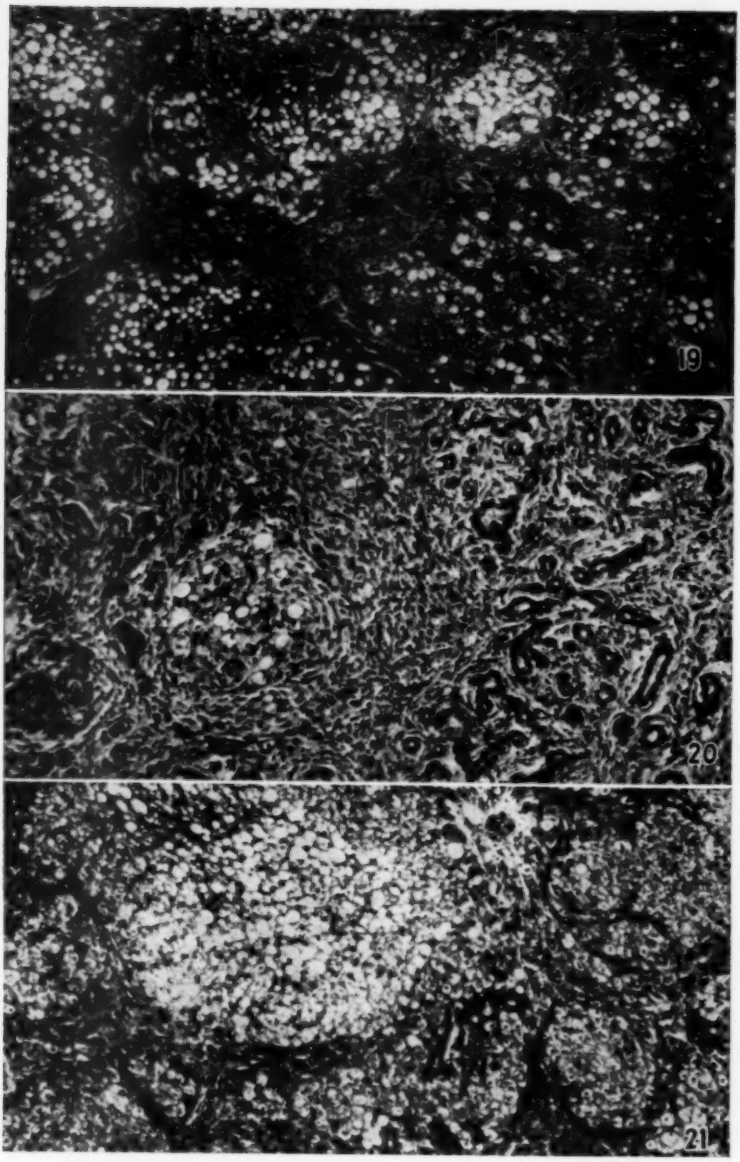
Fig. 14 (dog DE, see fig. 13).—The periportal location of the cellular infiltration and the early fibrosis in this liver are distinct. There is also the early reticular thickening of incipient fibrosis intralobularly. These reactions are not related to the fatty change, as the liver is not severely fatty. This liver contained only 10 per cent fatty acids. Hematoxylin and eosin; × 115.

Fig. 15 (dog D12, see fig. 9).—The connective tissue around the portal tract has undergone a peculiar change with eosinophilia and thickening. This reaction also extends into the surrounding parenchyma. The hepatic radicle related to this portal tract was apparently normal. Hematoxylin and eosin; × 170.

Fig. 16 (dog K, depancreatized 2.7 years prior to examination; maintained with insulin and a lean meat diet).—There is diffuse fibrosis throughout the very fatty parenchyma, with liver cells being replaced by fibrous tissue. The portal tract toward the lower left corner is only mildly fibrotic in comparison with the severity of the parenchymal changes. This liver contained 34 per cent fatty acids. Hematoxylin and eosin; × 70.

Fig. 17 (dog K, see fig. 16).—Diffuse interstitial active fibrosis in the subcapsular region. The young, cellular connective tissue is apparently spreading in all directions indiscriminately. There is early implication of the portal tract at the lower right edge of the picture. However, this portal tract shows no cellular infiltration, thickening or edema comparable with that depicted in figures 10, 13 or 14. Hematoxylin and eosin; × 70.

Fig. 18 (dog F20, fed a high fat diet for 36 weeks).—In the center of the picture the liver cells are replaced by dense bands of collagen fibers which disrupt the epithelial trabeculae. On all sides of this band of dense collagen, earlier stages of pericellular fibrosis are shown. Hematoxylin and eosin; × 112.



Figures 19-21

(See legends on opposite page)

seen in acute infections or toxic hepatitis in man. The impression gained is that some potent stimulus for hyperplasia of connective tissue has been imposed on the hepatic mesenchymal elements, which have responded diffusely and overrun the epithelial parenchyma. In many respects this lesion resembles that described by Karsner [11] as "intrahepatic cholangiolitic cirrhosis." But in our dogs, while centrolobular accumulation of bile can be detected as a complication in the late stages, the lesion does not appear to be induced by any detectable form of biliary infection or obstruction. It is possible, however, that some toxin or macromolecule of endogenous or exogenous origin may play a role in the causation of this diffuse hyperplasia of the connective tissue of the liver.[12]

In several livers the process, while progressing actively in some areas (judged by the extent and cellularity of the fibrous tissue), appears to be associated in other parts with the deposition of intralobular bands of eosinophilic collagen. Thus, in figure 18, above and below the center of the picture, active cellular interstitial fibrosis is proceeding, while in the center the early connective tissue is being supplanted by dense bands of collagen winding between and around the liver cells. With the advent of such a process, the hepatic trabeculae become disrupted, and portions of degenerating epithelial cords can be seen ensnared within the spreading connective tissue net (fig. 19, center).

Occasionally, the reaction depicted in figure 21 is encountered in livers undergoing interstitial fibrosis. Here chains of elongated, highly eosinophilic cells can be seen stretching indiscriminately through the parenchyma. The nuclei of these cells are small and hyperchromatic.

11. Karsner, H. T.: Am. J. Clin. Path. **13**:569, 1943.

12. Hueper, W. C.: Arch. Path. **33**:267, 1942. Gillman, J.; Gillman, T., and Gilbert, C.: South African J. M. Sc., to be published.

EXPLANATION OF FIGS. 19-21

Fig. 19 (dog F39, fed a high fat diet for 32 weeks).—The interstitial fibrosis is progressing actively and is well advanced. Highly cellular scars replace patches of hepatic epithelium. The joining of bands of cellular fibrous tissue has resulted in gross structural distortion. Hematoxylin and eosin; × 64.

Fig. 20 (dog F39, see fig. 19).—In this late stage of extremely active interstitial cirrhosis, a large area of liver parenchyma is replaced by highly cellular connective tissue which looks almost sarcomatous. The liver parenchyma is represented only by isolated groups of islands of fatty liver cells (left of center) and irregular groups of epithelial cells resembling proliferated bile ducts. Hematoxylin and eosin; × 130.

Fig. 21 (dog DE, see fig. 14).—A peculiar form of highly eosinophilic, cellular connective tissue stretches indiscriminately throughout the parenchyma. This is associated in one area (to left of center) with intrafatty fibrosis. Note also the eosinophilia with nuclear hyperchromasia of the liver cells just below center. Hematoxylin and eosin; × 85.

In some areas (see bottom center of fig. 21), the liver cells themselves seem to be undergoing a similar eosinophilic change. However, the nature of the reaction leaves little doubt that, whatever the genesis of this peculiar tissue, the result is diffuse interstitial fibrosis with structural distortion (fig. 21). This particular reaction resembles closely that described as occurring either periportally or around the hepatic radicles (compare fig. 21 with figs. 15 and 9). This reaction appears to be unrelated to the fatty change in the liver cells, although in figure 21 (top, left quadrant) another type of fibrotic change within the fatty areas seems to accompany this other peculiar form of eosinophilic fibrosis.

Structural distortion supervenes early with the progress of this interstitial type of hepatic fibrosis. At no stage during the evolution of this form of fibrosis do the lesions in the liver resemble those portrayed in figures 8, 12 and 14, for the fibrosis does not result in involvement of any localized portion of the lobule, as in the centrolobular and periportal types of fibrosis described. The end picture of this form of fibrosis when it is rapidly progressive is that portrayed in figures 19 and 20.

COMMENT

The four points of significance which emerge from this study of the genesis of hepatic fibrosis and cirrhosis in the dog are the following:

First, in the dog's liver fibrosis may originate periportally, between the parenchymal epithelial cells (interstitial) and/or around the radicles of the hepatic veins (centrolobular). These three forms of fibrosis can usually be distinguished easily in the early stages.

Second, some forms of fibrosis, especially the centrolobular form and perhaps the periportal and interstitial forms, appear to be related to the fatty change. But even in the centrolobular form, in which the association of the fibrosis with fat is usually more obvious, the fatty liver cells may remain quite intact and may not degenerate until the fibrosis is well advanced (fig. 3). In fact, the centrolobular fibrosis described here closely simulates, in pathogenesis, the reactions described in chronic cardiac decompensation in man [5] and in passive venous congestion of the liver in dogs.[4f] When this reaction occurs in the congested liver, it does not seem to be associated with, or preceded by, fatty changes in the liver cells. Possibly the circulatory changes occurring in a very fatty liver induce effects on the supporting reticulum similar to those occurring after long-standing venous congestion. Should this be the case, then attention must be devoted not only to the hepatocellular degeneration but also to the role of the vascular changes in the liver in the genesis of hepatic fibrosis. Attention has previously

been drawn to this aspect of hepatic fibrosis by Deysach [13] and later by Glynn and associates [4d] and by Himsworth and Glynn.[14]

The phenomena recorded to this point indicate that, as previously stated by Gillman and co-workers,[15] there are factors other than hepatocellular degeneration to be considered as being responsible for proliferation of the connective tissue of the liver. Special conditions seem to be necessary for hyperplasia of the connective tissue of the liver, for fibrosis does not necessarily follow even massive hepatocellular damage [16]; in fact, entire lobes of the liver may atrophy and disappear without any associated connnective tissue response.[15] Not only is this the case for the liver, but a similar observation appears to hold true for the pancreas and the salivary glands.[17] Thus, we have encountered reactions primarily in the interlobular septums of the dog's pancreas [18] or interstitial fibrosis in the human pancreas [15b] or even complete degeneration of the acinous tissue without any connective tissue reactions whatsoever.[19]

These observations concerning the liver and the pancreas of man and several laboratory animals indicate that special conditions are necessary for the development of fibrosis in these organs. It would seem that the parenchymal cells and the supporting tissues of these organs, while frequently reacting simultaneously, are nevertheless two independently reacting systems.

The third significant fact emerging from this study is that several different forms of fibrosis can occur in the livers of a group of dogs subjected to identical experimental procedures.[20]

In view of this last observation we are led to another conclusion—that the end stages of hepatic cirrhosis in dogs treated in different ways may be pathologically similar, even though the causation of the lesions and their genesis may be quite different. It seems that no conclusions concerning either the cause or the genesis of a cirrhotic process can be drawn from an examination of a single section of a liver taken at a single moment during the end stages of the disease.

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20. Chaikoff and others, footnotes 3 and 4 h.

SUMMARY

Three pathogenetically distinct forms of connective tissue hyperplasia or hepatic fibrosis have been induced experimentally in dogs: (1) centrolobular or peri-hepatic vein fibrosis; (2) periportal fibrosis; (3) diffuse interstitial or intralobular fibrosis.

The final pathologic pictures observed following the development of the three forms of fibrosis may be similar. The end picture of each is determined not only by the site of initiation of the fibrosis but also by the extent to which the liver is affected, the speed of development of the initial lesion and the degree to which the first type of fibrosis is complicated with focal necrosis, lobar or lobular hypertrophy or atrophy, and the supervention of additional forms of fibrosis.

While fibrosis, especially the centrolobular type, was frequently associated with severe fatty change of the hepatic epithelium or occasionally with central hemorrhagic necrosis, nevertheless it was also seen without being obviously related to hepatocellular damage.

Factors other than extensive degeneration or destruction of hepatic epithelium may play a critical role in determining the site of onset of hepatic fibrosis. Attention is drawn to the possible role of circulatory changes in the genesis of hepatic fibrosis. The relative independence in reactivity of the parenchymal cells and the supporting tissue of the liver is indicated.

Different forms of fibrosis can occur in animals treated in an identical fashion, and even in different parts of a single liver. No correlation could be established between the type of fibrosis or the terminal cirrhosis and the experimental procedure used to induce the hepatic changes.

ADENOMA OF CERUMINOUS GLAND IN THE DOG

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A CASE of adenoma of the ceruminous glands has a twofold interest. Only a few neoplasms of the ceruminous glands of man or animals have been reported and it is rare to find a tumor of cutaneous glands of either apocrine or eccrine type in which the growth can be traced to the secretory elements in distinction from the ducts. Three recent reviews of the subject are those of Warren and Gates,[1] Gates, Warren and Warvi [2] and Adler and Sommer.[3] Willis [4] found 1 dog with ceruminous adenoma among 204 dogs with various tumors. The tumor which is now reported has been reviewed by David Coffin,[5] of the Animal Angell Memorial Hospital of Boston, R. M. Mulligan,[5] of Denver, William Feldman,[5] of Rochester, Minn., Raymond O. Dart [5] and T. C. Jones,[5] of Washington, D. C. They have all agreed that it is an adenoma of ceruminous gland origin.[6]

In a seeing eye dog being treated for stubborn ulcerative otitis externa, two apposed growths developed in the cartilaginous portion of the external acoustic meatus approximately 2.5 cm. from the external orifice on the medial and lateral aspects of the canal. They were completely excised.

Two roughly spherical pieces of tissue partly covered by patches of thickened gray skin were received in 4 per cent formaldehyde solution. Each measured 1.2 by 1 by 0.7 cm. The cut surface showed grayish brown, firm, lobulated, glistening tissue dotted with yellowish flecks and reddish brown foci of hemorrhage.

Microscopically the neoplasm, which lay in the upper part of the corium, compressing but not joining the epidermis, appeared to arise from the secretory alveoli, although there were a few foci which suggested that the ducts also might be involved in the new growth. The tumor was composed of acinous and solid epithelial elements, with a predominance of the acinous component. These acini varied greatly in size. A few approached the dimensions of the normal alveoli,

From the Harvard Cancer Commission Laboratory of Pathology and the State Tumor Diagnosis Service.

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5. Personal communication to the author.

6. The specimen and the clinical history were provided by Paul R. Granholm, D.V.M., 348 Boston Post Road, Weston, Mass.

but most of them were larger and a few, five or six times the normal size, could be designated as cysts (fig. 1). The solid growth (figs. 2 and 4) was by comparison insignificant and appeared to result mainly from proliferation of cells within the alveoli rather than from infiltration of cells into surrounding stroma.

The cells of the tumor in the main resembled the normal secretory epithelium of the ceruminous glands (fig. 5). The commonest form was a cuboidal cell, either low or tall. The free surface was smooth and rounded, with the lipping characteristic of apocrine structures and occasionally a frayed border indicating active secretion. The nucleus tended to lie in the basal part of the cytoplasm. It was pale, and the rather large nucleolus was therefore prominent. The cytoplasm was slightly refractile and eosinophilic, sometimes pale but more often staining intensely. Products of secretion, such as fat, pigment globules and deeply acidophilic granules, were frequently a prominent feature of the cytoplasm (figs. 3 and 4). Amorphous golden brown material, eosinophilic granules and a rather amorphous hyaline substance lay free in the lumens of alveoli and in the stroma, where they attracted histiocytes. Special stains showed that the golden brown pigment granules contained iron, while the less refractile paler pigment, apparently intimately bound to the lipoid, did not.

Besides the obvious secretion of this tumor, the myoepithelial cells in a few of the well differentiated alveoli also indicated that it was derived from the secretory portions of the ceruminous gland. These myoepithelial cells were quite insignificant as compared with their appearance in the normal gland and were not recognized in the poorly differentiated alveoli. The more solid growth appeared to be the result of proliferation of cells lining alveoli rather than of invasion of the surrounding stroma. Most striking was a papilliform growth within dilated acini, the cells being tall columnar, with colorless or faintly basophilic cytoplasm. Squamous metaplasia of alveolar cells occurred frequently and sometimes progressed to complete keratinization, but epithelial pearls and intercellular bridges were not seen.

There was a moderately dense fibrous stroma showing areas of myxomatous change which produced the effect of an infiltrating growth. In the absence of a capsule a growth so variable in structure is apt to appear more cancerous than it is. The absence of invasion of blood vessels and lymphatic channels, the few mitotic figures and the active secretion suggest a benign tumor.

We have had an opportunity to examine four other tumors of the same type. These are enumerated:

Slides of 3 tumors of ceruminous glands from the files of the Registry of Veterinary Pathology of the Army Institute of Pathology were supplied by Brig. Gen. Raymond O. Dart, Medical Corps, and Major T. C. Jones, Veterinary Corps, United States Army:

Accession 183438, a tumor from the external auditory canal of a cat was submitted by Dr. R. F. Vigue, of Sanford, Maine.

Accession 185379, a tumor from the external auditory canal of a male Doberman pinscher was submitted by Dr. George M. Stewart, of Brentwood, Md.; this tumor was removed in January 1947; it recurred a month later and, following this excision, has recurred a second time.

Accession 185427 from a bull terrier 8 years old was submitted by Dr. S. W. Stiles, of Falmouth Foreside, Maine; there was no recurrence a year later.

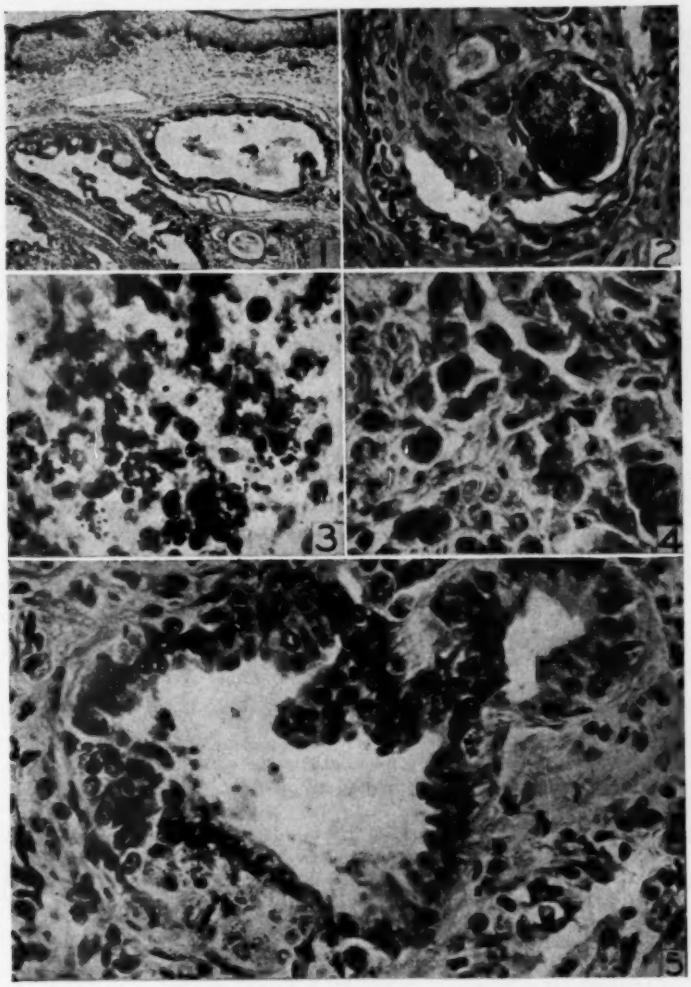

Fig. 1.—Cystically dilated glands with papilliform projections. × 50.
Fig. 2.—Squamous metaplasia in a duct. × 200.
Fig. 3.—Stromal histiocytes containing fat. Sudan IV; × 500.
Fig. 4.—Stromal histiocytes. × 500.
Fig. 5.—Gland showing "lipping" of the cells, denoting active secretion. Droplets of secretion lie free in the lumen. × 500.

A paraffin block from a fourth tumor [7] was supplied by Dr. R. M. Mulligan, professor of pathology at the University of Colorado School of Medicine. This tumor from a 15 year old male fox terrier was submitted by Dr. L. R. Phillips and Dr. B. S. Burkhardt, both of Lakewood, Colo.

Sections from these four tumors show essentially the same characteristics as the tumor presented in this report except that little solid growth occurred and there was no squamous metaplasia.

COMMENT

This canine ceruminous gland adenoma, though not encapsulated, appears to have represented an expansile rather than an actively infiltrating growth. The greater part was well differentiated and exhibited the functional characteristics of normal secreting acini of ceruminous glands.

It is of particular interest that some elements, namely, the papillary, almost mucinous type of columnar epithelium and the squamous keratinized masses of cells, which commonly occur in syringocystadenoma as a result of proliferation of epithelium of ducts, were in this case clearly derived from the secreting alveolar epithelium. It is rare indeed to find convincing evidence to implicate the secretory cells of cutaneous glands in a neoplastic process. This tumor therefore was unusual because it was a functioning tumor of ceruminous glands and because it demonstrated the fact that two different types of epithelium, secreting epithelium and lining epithelium of ducts, may through metaplasia produce the same form of tumor.

SUMMARY

A tumor of the ceruminous glands of a dog is here presented in detail. Four similar tumors of 3 dogs and a cat have been reviewed and are reported for the first time. Tumors of ceruminous glands have received little study. They are exceedingly rare in man, but several veterinarians have expressed the opinion that they are of common occurrence in the dog.

7. Dr. Mulligan's case bears the number 47R-304 under Research Grant C-380-R of the National Cancer Institute of the United States Public Health Service. Dr. Mulligan's recent review of 120 canine neoplasms (Arch. Path. **45**:216, 1948) does not include ceruminous gland tumors.

EFFECT OF EXPERIMENTAL THIAMINE DEFICIENCY ON THE HEART OF THE RHESUS MONKEY

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AND
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DURING the past several years progress in nutritional research has been rapid. With clarification of the chemical nature of essential food factors, particularly those of the vitamin B complex, it has become possible to study the influence of single deficiencies in the monkey with a degree of precision heretofore unattainable. It seems most timely to restudy the vitamin deficiencies in a primate in which the metabolic processes would be expected to approximate closely those of man. We have therefore undertaken systematic studies of the vitamin deficiencies in the rhesus monkey. The major lesions developing in the central nervous system and the heart as a result of thiamine depletion have been reported in abstract,[1] and the precise character and distribution of the degenerative lesions developing in the nuclear structures of the central nervous system have been detailed separately.[2] The present report is concerned primarily with the histopathologic changes seen in the heart muscle in thiamine deficiency.

METHOD

The diet used was a modification of the M-3 diet of Waisman and associates.[3] The composition of the diet, together with notes on the clinical behavior of the animals, biochemical observations on thiamine metabolism and influences on hemopoiesis have been reported.[4]

Particular mention should be made of the fact that of the 7 animals studied, 3 were subjected to two episodes of acute depletion and 2 others to three such episodes, the total periods ranging from one hundred to one hundred and seventy

From the Division of Pathology, University of California Medical School.

This investigation was supported by a donation from the California Fruit Growers Exchange and the A. B. Miller Fund.

1. Rinehart, J. F.; Greenberg, L. D., and Friedman, M.: Abstracted, Am. J. Path. **23**:879, 1947. Rinehart, J. F.; Friedman, M., and Greenberg, L. D.: Tr. Am. Neurol. A. **72**:174, 1946.

2. Rinehart, J. F.; Friedman, M., and Greenberg, L. D.: Arch. Path., to be published.

3. Waisman, H. A.; Rasmussen, A. F., Jr.; Elvehjem, C. A., and Clark, P. F.: J. Nutrition **26**:205, 1943.

4. Rinehart, J. F.; Greenberg, L. D., and Ginzton, L. L.: Blood **3**:1453, 1948.

days. One animal was maintained in a deficient state by administration of repeated suboptimal doses of thiamine for a total experimental period of sixty days. The other animal was examined at the end of a single depletion period of fifty days. There is no doubt that the procedure of producing recurrent depletion has augmented the pathologic changes.

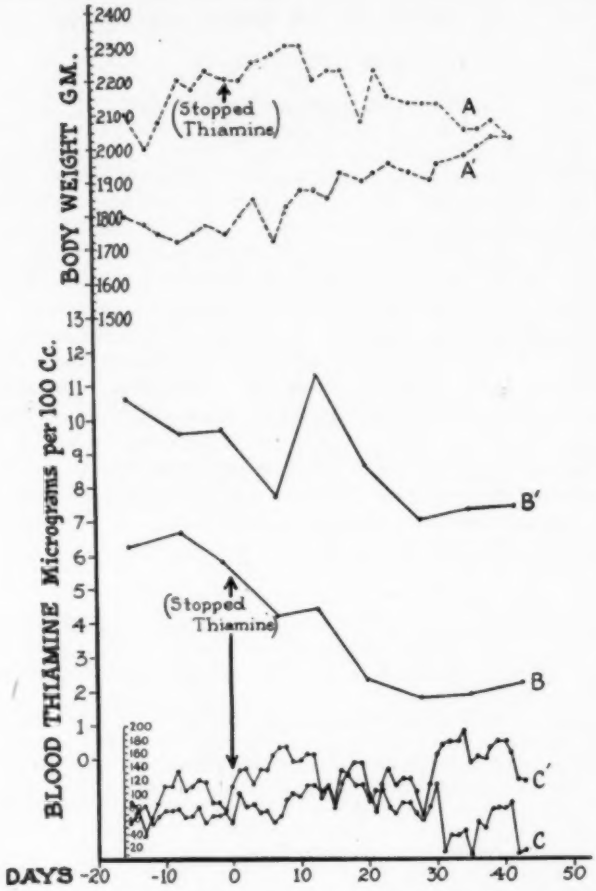

Fig. 1.—*A* and A^1 weight curves of a thiamine-deficient rhesus monkey and its control, respectively. *B* and B^1 blood thiamine levels of deficient monkey and control. *C* and C^1, food consumption curves of deficient monkey and control.

When thiamine is removed completely from the diet, the animals will show diminished consumption of food after two weeks with concurrent loss of weight.

This observation has been made repeatedly. Curves showing weight, food consumption and blood thiamine concentration for deficient and control animals are presented in figure 1. It is evident under the controlled conditions of observation that the animal is in fact beginning to suffer from thiamine deficiency after two to three weeks of depletion. While pathologic observations have not been made at this stage, we are confident that present methods of histologic examination would not reveal lesions. When the deficient state is prolonged, the animals become apathetic, inactive and progressively weaker. This is followed by ataxia and at times ptosis and tremors. Several animals have shown dyspnea on exertion when removed from the cage for examination. Even in such advanced states of depletion administration of thiamine will produce dramatic improvement in strength, locomotion, appetite and reactivity. Control animals maintained on the same basic diet with supplements of thiamine have remained in vigorous good health.

We have recently reported a sensitive method for the determination of the thiamine levels of blood and tissues.[5] The blood thiamine of the monkey fed an adequately supplemented diet ranges from 5.5 to 10 micrograms per hundred cubic centimeters, while in the depleted animal it is from 2 to 4 micrograms (fig. 1).

PATHOLOGIC CHANGES IN THE HEART

Gross examination of the hearts showed dilatation of the right auricle and ventricle in 5 of the 7 animals and at times some dilatation of the left ventricle (fig. 1).

Microscopic examination revealed that significant cardiac lesions had developed in 4 of the 7 animals. The lesions were of two types. One consisted of focal necrosis of the heart muscle as shown in figures 2 *A* and *B*. This lesion was of variable extent. It appeared to represent slow degeneration or necrobiosis of muscle fibers rather than acute necrosis. There were reactive hyperplasia of the reticular stromal cells and some leukocytic infiltration. In the animal showing the most extensive necrosis of this type, the lesion was chiefly beneath the endocardium and of such extent that had it healed it probably would have occasioned some subendocardial fibrosis. The other type of cardiac lesion was seen in the same 4 animals. Although somewhat elusive, it was perhaps more distinctive of thiamine deficiency. It consisted of swelling and development of large clear areas in the cytoplasm of certain subendocardial muscle fibers, accompanied by hypertrophy and hyperchromatism of associated nuclei. At times there appeared also to be a degree of interstitial edema. The character of the lesion is well shown in figures 2 *D* and *E*. The lesion was most frequently seen in the left ventricle but was observed also in the right ventricle. The size and the distribution of abnormal fibers leave little doubt that they were part of the specialized myocardial fibers of the conduction system. We have not encountered lesions of either type in the auricles.

5. Greenberg, L. D., and Rinehart, J. F.: Proc. Soc. Exper. Biol. & Med. **59**:9, 1945.

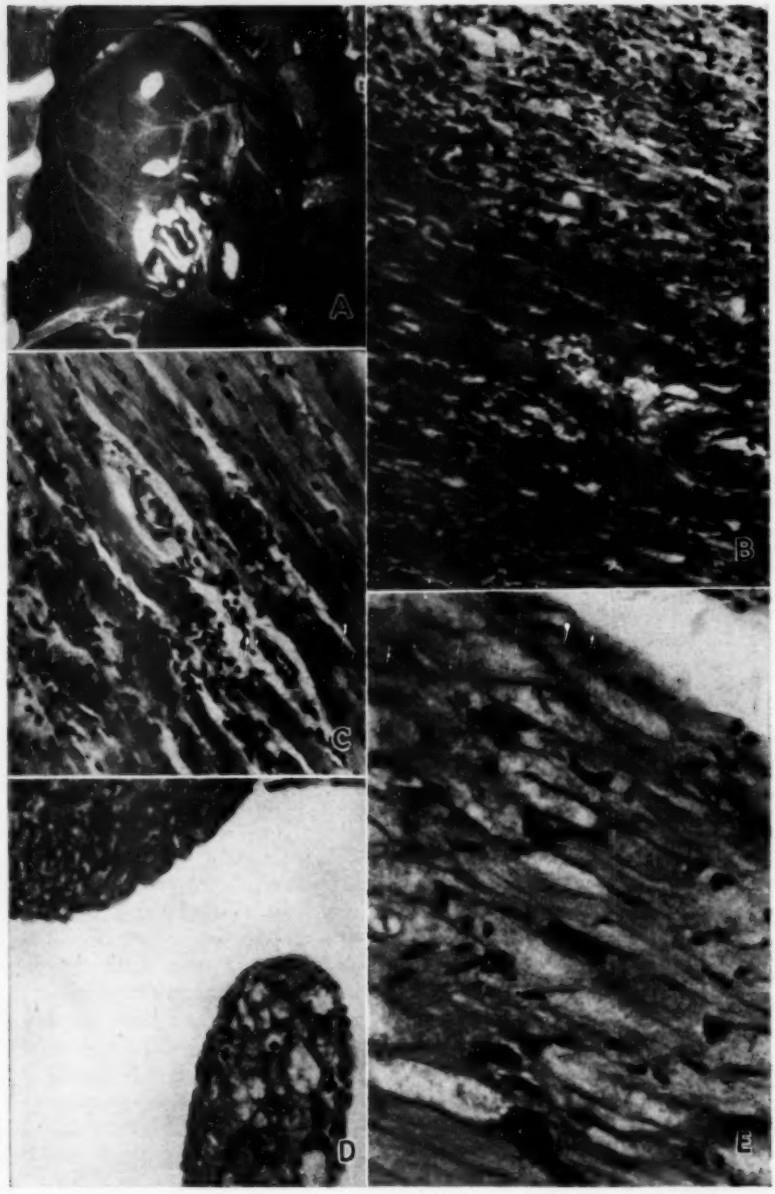

Fig. 2.—*A*, gross appearance of the heart in acute recurrent thiamine deficiency. Note dilatation of both ventricles, particularly of the right. *B*, extensive subendocardial degeneration and necrosis of heart muscle; × 205. *C*, small focus of myocardial necrosis; × 205. *D*, striking "hydropic" swelling of subendocardial muscle fibers of the conduction system; × 205. *E*, myocardial fibers beneath the endocardium; × 410. Note irregular hyperchromatic nuclei and pallor and swelling of cytoplasm.

COMMENT

That a functional cardiovascular defect occurs in thiamine deficiency in man and animals is well established. The exact nature of this defect, however, has not been clarified, and the morphologic manifestations have remained somewhat obscure. Follis and associates [6] recently reviewed pertinent literature and reported focal necroses of the myocardium of swine subjected to thiamine deficiency. Van Etten, Ellis and Madsen [7] also described such lesions of pigs given a diet supplemented by sulfite-treated liver and whey. Lesions of similar character have been observed in pigeons by Swank [8] and in 3 of 14 dogs subjected to thiamine deficiency by Swank, Porter and Yoemans [9] Porto and de Soldati [10] and de Soldati [11] reported myocardial "infarcts" of a dog subjected to thiamine deficiency and hydropic degeneration of myocardial and conduction fibers of other animals. In de Soldati's monograph focal necroses of the heart muscle of the thiamine-deficient dog are described and illustrated, as well as perinuclear vacuolar degeneration of muscle fibers, including those of the conduction system. Focal myocardial necroses associated with hemorrhage were noted by Evans, Carlson and Green [12] in foxes dying of Chastek paralysis, which is almost certainly dominantly a thiamine deficiency syndrome. They also noted enlargement of nucleoli and intracellular edema of cardiac muscle cells in some instances. More recently Ashburn and Lowry [13] observed myocardial necroses in rats subjected to prolonged thiamine deficiency. These lesions were dominantly in the auricles. Forty-seven of 60 rats had "necrosis of muscle fibers, cellular infiltration and proliferation or evidence of previous damage, such as decreased number or absence of muscle fibers and slight to moderate fibrosis." Similar lesions were seen in the ventricular myocardium in only 7 rats, and in these the involvement was minimal.

It appears that focal necroses of the myocardium have been observed in many species of animals subjected to thiamine deficiency. The vacuolar or hydropic degeneration of the fibers of the conduction system appears to be more distinctive of thiamine deficiency. Lesions

6. Follis, R. H., Jr.; Miller, M. H.; Wintrobe, M. M., and Stein, H. J.: Am. J. Path. **19**:341, 1943.

7. Van Etten, C.; Ellis, N. R., and Madsen, L. L.: J. Nutrition **20**:607, 1940.

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11. de Soldati, L.: Los trastornos circulatorios de la avitaminosis B_1, Buenos Aires, El Ateneo, 1940.

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13. Ashburn, L. L., and Lowry, J. V.: Arch. Path. **37**:27, 1944.

of this type in man have been recorded. Thus Wenckebach [14] described and illustrated an apparently identical pathologic change in his classic study of the beriberi heart. Weiss and Wilkins [15] were responsible for directing attention to the not infrequent occurrence of beriberi heart disease in the United States. They made pathologic examinations in a few cases and recorded "hydropic" degeneration of muscle fibers and illustrated changes of subendocardial conduction fibers that are analogous to those detailed by Wenckebach and similar to those described in this report. Weiss and Wilkins questioned the specificity of the "hydropic degeneration" because such lesions were said to be seen in other patients not known to have thiamine deficiency.

SPECIFICITY AND SIGNIFICANCE OF THE LESIONS

The focal myocardial necroses, although frequently encountered in experimental thiamine deficiency, can scarcely be considered specific. As pointed out by Follis, analogous lesions have been seen in potassium deficiency and in scurvy. It is also noteworthy that such focal necroses have not been mentioned in descriptions of the human beriberi heart. The peculiar "hydropic degeneration" of the conduction fibers is, we believe, in its fully developed stages quite distinctive. It has been demonstrated in 4 of the animals of this study and corresponds to the lesion that has been observed in human deficiency. It should be borne in mind that the conduction fibers are somewhat larger than other heart muscle fibers and normally show larger nuclei and paler cytoplasm. In a certain sense the pathologic change is an exaggeration of a normal cytologic feature of the conduction fiber. The lesion is consequently somewhat elusive and must be well developed to be characteristic. A somewhat similar appearance was noted in 1 animal subjected to folic acid deficiency but not in controls or other animals undergoing equally prolonged periods of inanition incident to other deficiencies.

While both lesions are definite, it is problematic whether they are fully responsible for the circulatory defect in thiamine deficiency. Both Wenckebach and Weiss and Wilkins have presented evidence that there is a peripheral arteriolar dilatation in beriberi which is so marked that it acts similarly to an arteriovenous shunt. The latter have shown that the circulation time is shortened in beriberi and that there is decreased peripheral utilization of oxygen. Weiss suggested that this phase of the circulatory defect is probably related to disturbances of nerve functions, possibly in the central nervous system. Our observations of widespread and significant lesions in many of the nuclear structures of the central

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15. Weiss, S., and Wilkins, R. W.: Ann. Int. Med. **11**:104, 1937.

nervous system in thiamine-deficient monkeys [2] lend strong support to this concept. Unfortunately, we did not study the circulation time or the peripheral utilization of oxygen in our animals.

The only other recorded experiments in which thiamine deficiency was produced in the monkey and which may be considered to be uncomplicated with other deficiencies are those recently reported by Waisman and McCall.[16] They noted decreased consumption of food, loss of weight, muscular weakness, loss of reflexes, convulsions, incoordination, increasing cachexia and signs of cardiac insufficiency as the clinical manifestations of thiamine deficiency in the rhesus monkey. The blood "pyruvic acid" level was sharply elevated in the thiamine-deficient animal. Electrocardiographic tracings showed lowered heart rate, decreased amplitude of the R wave and inversion of the T waves. The few electrocardiographic tracings which we have made are similar. Histopathologic studies were not reported.

It would seem desirable to determine the concentration of thiamine in the blood and possibly skeletal muscle of patients whose symptoms suggest thiamine deficiency and to correlate studies of the blood and tissue contents of thiamine with histopathologic changes in cases of otherwise unexplained circulatory failure such as might be encountered in the coroner's jurisdiction. Likewise, careful physiologic studies of the thiamine-deficient monkey should serve to clarify the nature of the significant circulatory defect.

SUMMARY

Rhesus monkeys subjected to recurrent episodes of thiamine deficiency show dilatation of the right side of the heart and focal necroses in the heart muscle which are analogous to lesions described in many other species. A second and probably more distinctive lesion is a peculiar degenerative change occurring in the fibers of the conduction system, manifested by irregular hypertrophy of nuclei and "hydropic degeneration" of the cytoplasm. It is problematic whether these lesions are fully responsible for the circulatory defect of thiamine deficiency. The clinical observations of marked arteriolar dilatation in human thiamine deficiency suggest the important influence of a vasomotor factor. The finding of widespread degenerative changes in the nuclear structures of the brain stem in the animals of this experiment [2] lends support to this concept.

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Case Reports

ONCOCYTOMA OF THE PAROTID GLAND

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HAMPERL[1] originated the term "onkocyte," although he gave credit to Shaffer for having first described these cells in the salivary glands, the pharynx, the trachea and the esophagus as "granular swollen cells." The name "onkocyte" was suggested since the cell is characterized by the constitution of its cytoplasm and consequent enlargement. Further investigation concerning this type of cell revealed that certain tumors of the salivary glands were made up largely of oncocytes.[2] Ackerman[3] gathered 7 well documented cases of oncocytoma of the salivary glands from the literature and added 1 of his own. The case reported here is one of a "mixed" type of tumor in which the epithelial component is made up of oncocytes.

REPORT OF CASE

A Negro man aged 67 was seen in the tumor clinic in January 1948. Beneath the right ear there was a painless firm lump which had gradually increased in size since the patient first noticed it two years previously. It was approximately 5 cm. in diameter and was not attached to skin or bone, and was movable.

The tumor was widely excised, the specimen including a generous portion of apparently normal parotid gland. It was 7 by 5 by 3.5 cm. and weighed 35 Gm. The tumor was irregularly rounded and rather firm and appeared fairly well encapsulated. On sectioning, the surface was gray-brown and homogeneous except for two small areas which were soft and cystic. Numerous blocks were selected for microscopic examination and stained with hematoxylin and eosin.

The tumor had a thick, though incomplete, connective tissue capsule. For the most part, the cells were uniform and arranged in columns and pseudoacinous fashion without formation of true glands or ducts. The groups of cells were separated by delicate strands of connective tissue. The individual cells were large, with abundant bright acidophilic granular cytoplasm. The nuclei were round, with evenly distributed chromatin; nucleoli were quite prominent. The cells had a striking resemblance to hepatic or adrenal cells.[3] No mitotic figures were found. In a few areas there were groups of smaller cells that tended to form ducts; these areas contained more stroma, which varied from hyaline through mucoid to cartilaginoid. The portion of the gland adjacent to the tumor was normal.

From the Department of Pathology, University of Louisville School of Medicine.

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2. Hamperl, H.: Ztschr. f. mikr.-anat. Forsch. **27**:26, 1931.

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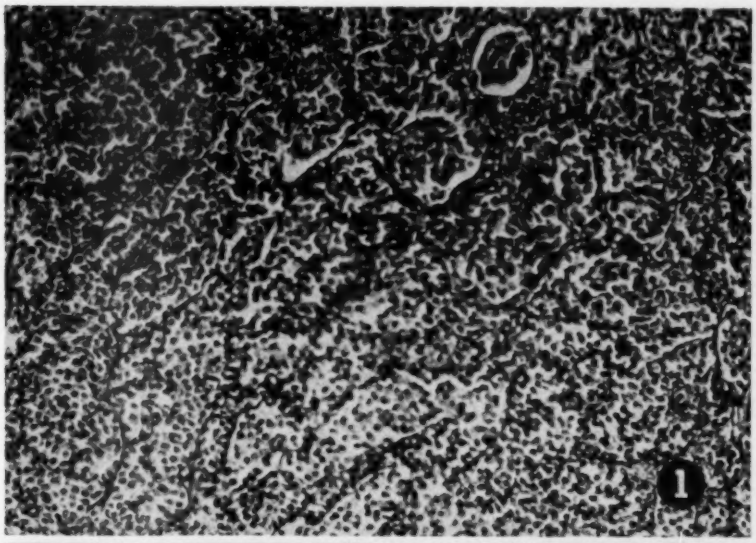


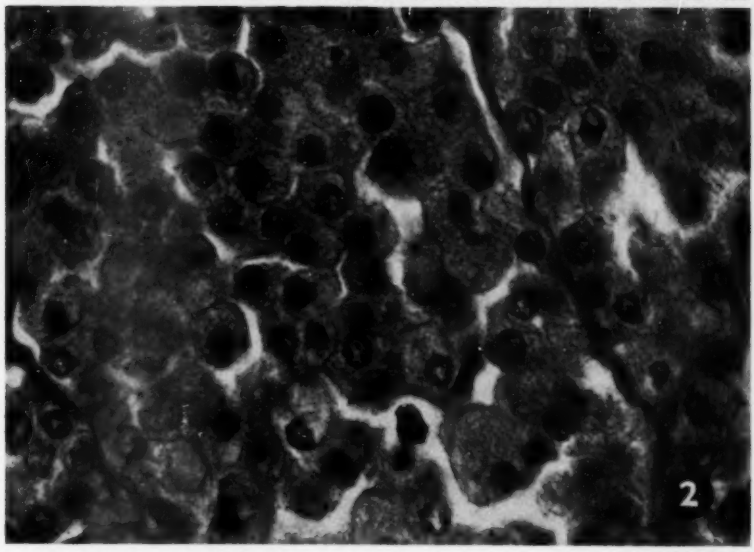


Fig. 1.—Low magnification showing the alveolar arrangement of the cells and the paucity of stroma.

Fig. 2.—High magnification showing the large cells with superficial resemblance to adrenal or liver cells.

COMMENT

Oncocytes are epithelial cells characterized by a peculiar honeycombed acidophilic granular cytoplasm. They resemble the cells of the parent organ but are much larger, those found in the parotid gland having rather a close resemblance to hepatic or adrenal cells. Their exact origin is not known; they seem to represent a new type or form of an already differentiated cell,[4] or what amounts to redifferentiation. These cells appear with sexual maturity and increase with advancing age, which suggests that the peculiar appearance is due to senility of the cell. Hamperl [2] found signs of cell proliferation but always with amitotic cell division, whereas in physiologic regeneration of salivary glands mitoses are observed. In addition to the salivary glands, oncocytes have been demonstrated in the pancreas, the parathyroid glands, the hypophysis, the thyroid gland, the fallopian tubes, the liver, the testes [4] and the bronchi.[5] Stout [5] established that oncocytes are present in the bronchi of adults and not present in fetal bronchi. He considered that these cells may possibly be the stem cells of bronchial adenoma.

Without entering into the controversy as to the origin of the so-called "mixed" tumors I believe it is possibly of some significance that in the tumor reported here the small areas of cartilaginoid stroma occurred only in a few regions, where the cells were smaller and tended to form ducts, and was conspicuously absent from the bulk of the tumor, in which the epithelial component was purely oncocytic.

The interval since excision of this tumor is too short for one to predict the ultimate outcome, but at the time of writing there is no recurrence and no histologic evidence of cancer. This is in accord with the previously reported cases, in all of which the tumor behaved as a benign neoplasm.[6]

SUMMARY

Oncocytoma of the parotid gland is a rare tumor with a striking, easily recognized histologic structure. In the case reported here the tumor contained small cartilaginoid areas similar to those found in the so-called "mixed" tumors.

4. Hamperl, H.: Virchows Arch. f. path. Anat. **298**:327, 1936.
5. Stout, A. P.: Arch. Path. **35**:803, 1943.
6. Hamperl, H.: Virchows Arch. f. path. Anat. **300**:46, 1937. Ackerman.[8a]

Notes and News

Appointments, etc.—George J. Rukstinat has been appointed professor of clinical pathology in Loyola University, Chicago.

P. R. Beamer, Washington University Medical School, St. Louis, has been appointed professor of microbiology and immunology and associate professor of pathology at the Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.

Hugh W. Jones, formerly chief of pathology, Walter Reed General Hospital, Washington, D. C., has become director of the department of pathology and clinical laboratories of the Mason Clinic in Seattle, Wash.

Death.—Felix d'Herelle, well known for his work on bacteriophage and his writings in English and French on immunity in infectious diseases, died in Paris, Feb. 22, 1949. He was born in 1873, in Montreal, where he received his M.D. degree. He was chef de laboratoire in the Pasteur Institute in Paris from 1914 to 1921. During this time and later he frequently served on special missions to various countries and was professor of protobiology in Yale University from 1928 to 1933, when he founded his own laboratory in Paris.

Training for Cancer Research.—The University of California Medical School announces a postgraduate course in the medical aspects of nuclear energy, August 29 to September 3, at the Medical Center, San Francisco. Joseph G. Hamilton, director of the Crocker Laboratory, University of California, will be chairman of the course. A detailed program will be mailed on request addressed to: Stacy R. Mettier, Head of Postgraduate Instruction, Medical Extension, University of California Medical Center, San Francisco 22.

The Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, has established a residency for a minimum of six months for training of pediatricians in neoplastic diseases of childhood. Address: Director of Pediatric Service, Memorial Hospital, 444 East Sixty-Eighth Street, New York 21.

The American Cancer Society announces the availability of the Damon Runyon Clinical Research Fellowships which it administers on recommendation of the National Research Council. Open to men and women with the M.D. degree, the fellowships will, in most cases, provide a period of training in a hospital under the guidance of a qualified clinical investigator but may also be awarded for training in a basic science, provided that such training is directed toward preparing the fellow for clinical cancer research. The annual stipend may vary from $2,500 to $6,000; in most instances it will not exceed $4,000. Applications may be submitted at any time and should be addressed to the Executive Secretary, Committee on Growth, National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

Training for Teaching and Research.—The American College of Physicians announces that a limited number of fellowships in medicine will be available from July 1, 1950 to June 30, 1951. These fellowships are designed to provide an opportunity for training in research either in the basic medical sciences or in the application of these sciences to clinical investigation. They are for the benefit of physicians who are in the early stages of preparing for a teaching and investigative

career in internal medicine. The stipend will be from $2,200 to $3,000. Application forms will be supplied on request to the American College of Physicians, 4200 Pine Street, Philadelphia 4, and must be submitted in duplicate not later than Oct. 1, 1949.

Society News.—Newly elected officers of the Alabama Association of Pathologists for the year 1949-1950 are J. A. Cunningham, president, J. S. P. Beck, vice president, R. D. Baker, member of executive committee and A. E. Casey, secretary-treasurer, Birmingham.

The American Association of Pathologists and Bacteriologists at its recent meeting in Boston elected Drs. Shields Warren, Alan R. Moritz and Sidney Farber, all of Boston, president, secretary and treasurer, respectively.

Revised Edition of Motion Picture Reviews Now Available.—The Committee on Medical Motion Pictures has completed the first revised edition of the booklet entitled "Reviews of Medical Motion Pictures." It now contains all the film reviews published in *The Journal of the American Medical Association* to January 1, 1949. It also includes a classified table of contents, as well as a list of motion pictures available through the Motion Picture Library, American Medical Association.

The purpose of the reviews is to provide a brief description and evaluation of motion pictures which are available to the medical profession. Each film is reviewed and commented on by competent authorities.

Copies are available on request from:

Committee on Medical Motion Pictures
American Medical Association
535 North Dearborn Street
Chicago 10, Illinois

Books Received

PSYCHODYNAMICS AND THE ALLERGIC PATIENT. By Harold A. Abramson, M.D., associate physician for allergy, Mount Sinai Hospital, New York; consulting physician for allergy, Sea View Hospital, Staten Island, N. Y.; assistant professor of physiology, Columbia University, New York. Panel Discussion: Rudolph L. Baer, M.D.; Ethan Allan Brown, M.D.; Hal M. Davison, M.D.; O. Spurgeon English, M.D.; Frank Fremont-Smith, M.D.; J. A. P. Millet, M.D.; M. Murray Peshkin, M.D.; Homer E. Prince, M.D.; Sandor Rado, M.D.; Edward Weiss, M.D. An official publication of the American College of Allergists. Pp. 81, with 6 illustrations. Price $2.50. St. Paul and Minneapolis, Minn.: Bruce Publishing Company, 1948.

This book is a significant contribution to the growing literature dealing with the psychosomatic aspect of allergic disorders. It is particularly important because it is directed to the attention of the practicing allergist and succeeds in demonstrating in a conclusive manner the significance of emotional factors in the total etiologic picture of the allergic patient.

The book is a report of a panel discussion on the subject "Psychodynamics and the Allergic Patient" held under the auspices of the American College of Allergists at its annual meeting in Atlantic City, N. J., June 8, 1947. It contains Abramson's paper on the subject as well as the discussion by members of the panel. Although the paper and the discussion do not take into consideration the more specific emotional dynamics relating to the various allergic manifestations that have been worked out and reported by psychoanalysts, the book does call the allergist's attention to the need for a revision of the attitude toward both the theory and the practice of the treatment of the allergic patient, indicating that immunologic methods are not enough and that advantage must be taken of the advances made in psychoanalytic psychology.

Abramson's paper is also interesting from a historical point of view. He shows that many observers going back hundreds of years were aware of the emotional factors in hay fever and asthma. The members of the panel contributed excellent discussions on the subject and these are properly recorded in the book. In addition to emphasizing the psychosomatic approach, the problem of who is to treat the allergic patient is touched on. Another topic of related interest discussed was the manner of how much and what kind of training in the psychosomatic approach should be given to the allergist. The book is a fine beginning and fills a need in the library of the physician and particularly the allergist.

FETAL AND NEONATAL DEATH: A SURVEY OF THE INCIDENCE, ETIOLOGY AND ANATOMIC MANIFESTATIONS OF THE CONDITIONS PRODUCING DEATH OF THE FETUS IN UTERO AND THE INFANT IN THE EARLY DAYS OF LIFE. By Edith L. Potter, M.D., Ph.D., associate professor in the department of obstetrics and gynecology, University of Chicago, and pathologist at the Chicago Lying-In Hospital, and Fred L. Adair, M.D., Mary Campau Ryerson professor emeritus, University of Chicago. Pp. 173, with 38 illustrations and 19 tables. Price $3.75. Chicago: The University of Chicago Press, 1949.

The first edition of this book appeared in 1940. In this, the second, edition the authors have added pertinent subject matter on the Rh factor and its influence on the fetus and the newborn child, and on the effects of maternal rubella (German measles) on the fetus.

In the preface Dr. Potter emphasizes that in the nine years which have elapsed since the book was first written, there has been "a widespread increase in the amount of attention centered on pregnant women and on the fate of their unborn and newly born offspring." "In spite of the fact that there were almost six hundred thousand more births in 1945 than in 1937 . . ., there were about a thousand fewer stillbirths and fourteen thousand fewer deaths under one month of age. The combined rate per 1000 live births had dropped from about 66 in 1937 to 48 in 1945."

The book is divided into five chapters. Chapter I deals with the normal fetus and infant. This chapter includes authoritative information on fertilization and early development, measurements and weights. This is followed by a detailed discussion of the developmental anatomy of each organic structure of the body during fetal life and in the newborn infant. Chapter II discusses the postmortem examination—first, general considerations, followed by the special technics necessary to successful and adequate examination of each body system. A well written chapter on the examination of the skull and brain of the infant should appeal to pathologists in training and to those unfamiliar with the modifications in technic which are necessary to demonstrate hemorrhage and dural tears without the introduction of artefact produced by the examiner. The method of opening the heart so that cardiac defects will not be missed is also well presented. Chapter III is a survey of the principal causes of fetal and neonatal death, and deals with malformations, anoxia, birth trauma, toxemia and infections, in a general manner, pointing out the special anatomic features of each with comparisons of later life where indicated. Chapter IV continues the discussion of causes of fetal and neonatal death under the title "Special Pathology." In this, the pathology of the organic systems of the body are reviewed in greater detail. Included in this chapter is a short discussion of the Rh factor and its effects on the fetus and the newborn infant, attention being given also to the hemorrhagic diseases. Chapter V presents the statistical data pertaining to births, maternal deaths, infant deaths and stillbirths. It includes many tables and charts as a visual aid to those interested in a statistical study of the problem.

The book is clearly written. The illustrations and tables aid in clarifying the special features of morbidity and mortality of the fetus and the newborn infant. It is based chiefly on Dr. Potter's vast experiences at the Chicago Lying-In Hospital and elsewhere in the city of Chicago. It is complete in its subject matter, although brief in detailed descriptions of the anatomic pathology. The reviewer has derived considerable satisfaction and information from this little book, and has found it a valuable guide in the teaching of residents. If there were a tendency to be critical, it would be only the wish that some day Dr. Potter might find it desirable to do an exhaustive work on the special pathology of the fetal and newborn period of life.

METHODEN DER PATHOLOGISCHEN HISTOLOGIE. By Frédéric Roulet, A. O. professor of pathologic anatomy; prosector of the pathologic institute of the University of Basel. Pp. 567, with 20 illustrations. Price, 15.40 dollars br. bound. Vienna, Austria: Springer-Verlag, 1948.

For a generation German pathologists had been taught histologic methods according to the classic "Pathologisch-histologischen Untersuchungsmethoden" of Schmorl. In those days "Schmorl" was found in every laboratory. Roulet's book, the reviewer believes, is destined to become "Schmorl's" successor. In this scholarly manual Roulet presents the material with special regard for the needs of the inexperienced student. He is detailed in his explanations and generous with

his references; a paragraph of citations of the more important relevant literature is added to each chapter. The technical methods described include the recent advances in the field of histochemistry and fluorescent microscopy. Phase-contrast microscopy is also discussed. An interesting chapter, and important for those pathologists who "review" histologic slides for diagnosis, is that which deals with the restaining of finished slides and the removing from such slides of certain impurities. Attention is paid to means of transporting material to distant places for histologic examination. There is also an extensive chapter on the demonstration of bacteria, protozoa and certain elementary bodies. Any one interested in histologic technic will find this manual an excellent reference book for procedures ranging from the simplest to the most elaborate specific determinations.

HOW TO BECOME A DOCTOR. A COMPLETE GUIDE TO THE STUDY OF MEDICINE, DENTISTRY, PHARMACY, VETERINARIAN MEDICINE, OCCUPATIONAL THERAPY, CHIROPODY AND FOOT SURGERY, OPTOMETRY, HOSPITAL ADMINISTRATION, MEDICAL ILLUSTRATION, AND THE SCIENCES. By George R. Moon, M.A., examiner and recorder, University of Illinois Colleges of Medicine, Dentistry and Pharmacy. Pp. 131. Price $2. Philadelphia and Toronto, Canada: The Blakiston Company, 1949.

PATHOLOGIE DES KOHLEHYDRATSTOFFWECHSELS. By Prof. Dr. E. Frank, director of the II. medizinischen Klinik der Universität Istanbul. Cloth. Pp. 342, with 20 illustrations. Price 24 Swiss francs. Basel, Switzerland: Benno Schwabe & Co. Verlag. New York: Grune & Stratton, Inc., 1949.

This volume is a thoughtful consideration and an attempted synthesis of the physiologic, biochemical and clinical aspects of the various disturbances of carbohydrate metabolism. The author is an experienced older clinician, whose interest in the pathologic physiology of diabetes and other disturbances spans a considerable period of time. Nevertheless, it may be said to his credit that his outlook has advanced with the times and that his point of view, though conservative, is well balanced. Although written by a clinician, the book is not primarily clinical in nature. It will therefore be of greater interest to the physiologist and to the specialist in diabetes than to the general student of internal medicine.

EXPERIMENTAL IMMUNOCHEMISTRY. By Elvin A. Kabat, Ph.D., associate professor of bacteriology, College of Physicians and Surgeons, Columbia University and the Neurological Institute, New York, and Manfred M. Mayer, Ph.D., associate professor of bacteriology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore. With a foreword by Michael Heidelberger, Ph.D., professor of biochemistry, College of Physicians and Surgeons, Columbia University, and chemist to the Presbyterian Hospital, New York. Pp. 567, with 88 illustrations. Price $8.75. Springfield, Ill.: Charles C Thomas, Publisher, 1948.

This useful book, which is less than a text but more than an abridged compilation, may be described as a somewhat elaborated compendium of some principles and technics that are being used in the progress of immunology toward the status of a "science." Applicable principles and methods have been harvested from the fields of physics, organic, biologic and analytic chemistry, and even from mathematics. But, although mathematics should be applied, there are, and will be for a while, too much relativity and too many simplifications to ask the "Queen of the Sciences" to certify an authentic exactitude to many features of the antigen-antibody reaction.

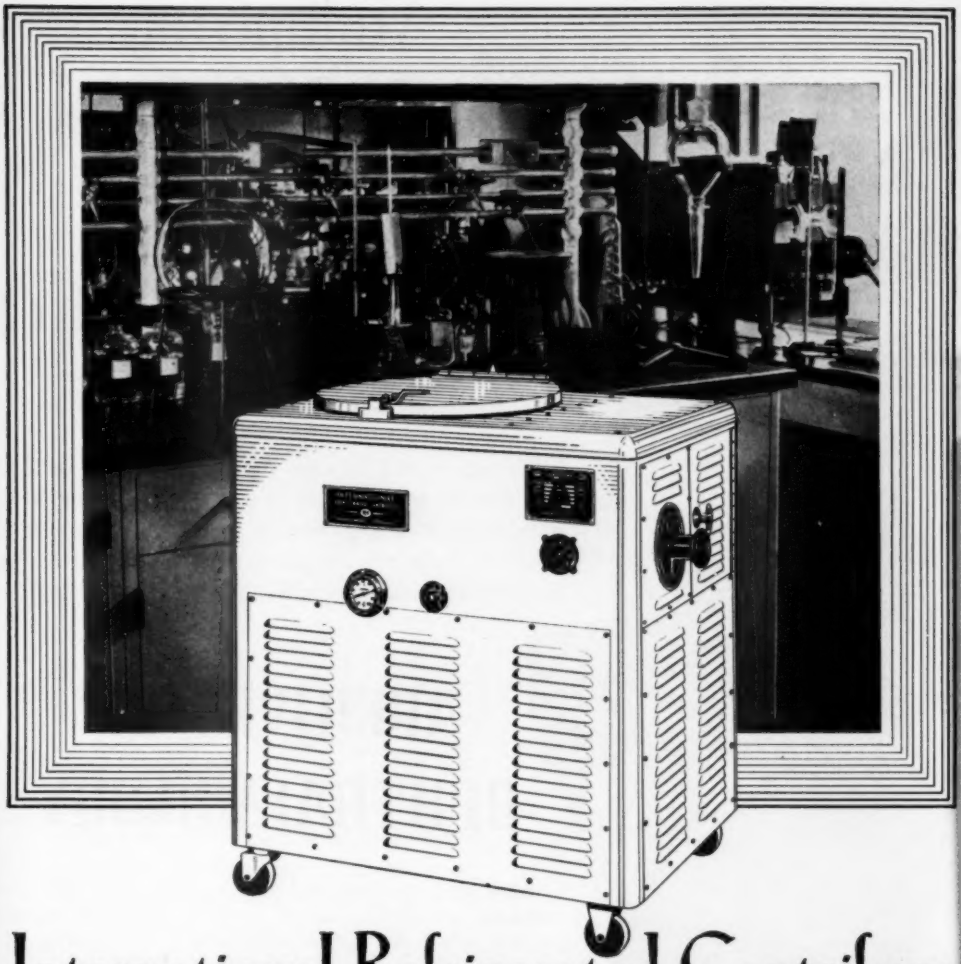
This book details the conventional immunologic methods and includes descriptions of modern (current) devices for quantitative measurements. The number of special procedures is too long to list here, but in addition to the Kjeldahl, van Slyke and Folin-Cicoalteau methods (to which nominal list the Heidelberger-Kendall-MacPherson method should be added) the authors discuss unnamed methods involving electrophoretic and ultra-centrifugal analyses, diffusion, optical rotation, solubility and ultraviolet ray absorption spectrums.

The section on "preparations" has eighteen chapters, each dealing with purification of natural antigens or with couplings that involve azo-, acetyl-, malonyl-, phosphoryl-, phenylureids-, carbobenzoxy- and other acid-chloride linkages with antigens.

In a short appendix the mechanics of centrifugation, colorimetry, calibration, preparations of antigens and injection into animals are considered.

The "preview," a humorous, flavorous and penetrating foreword by Heidelberger —who quite unconvincingly denies paternity of this volume—reemphasizes the still too widely unrealized usefulness of immunochemical methodology for discriminatingly qualitative and delicately quantitative study of the externally structural (and dependent) properties of compounds ranging from the fairly simple to the most highly complex.

This making of a book by two intimately experienced, alert, critical and vigorous young men merits high commendation.



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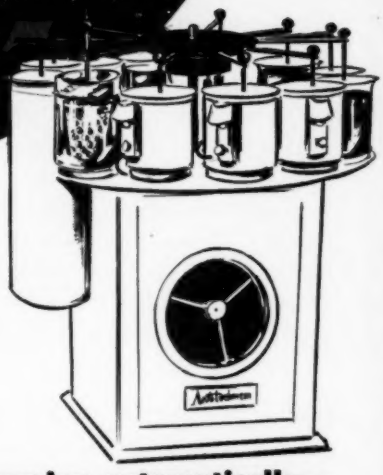

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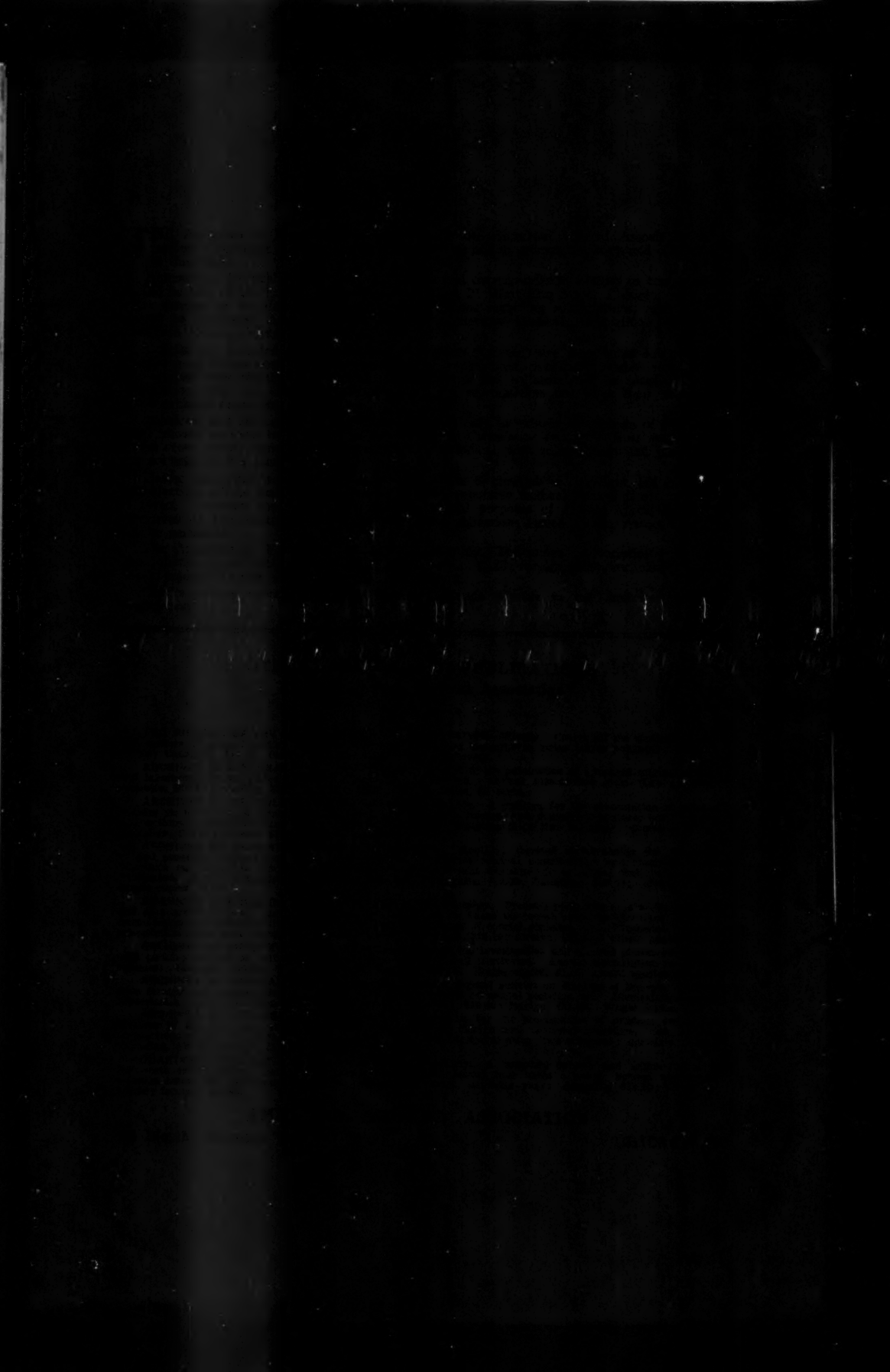
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